Astringency and other oral sensations: biological sources of individual variation and association with food and beverage behaviour.

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ABSTRACT

Orosensory perception strongly influences liking and consumption of foods and beverages. This thesis examines the influence of biological sources of individual variation on the perception of prototypical orosensory stimuli, food liking, self-reported alcohol liking and consumption, and indices of health. Two orosensory indices were examined: propylthiouracil (PROP) responsiveness, a genetically-mediated index of individual variation associated with enhanced responsiveness to orosensory stimuli often expressed as PROP taster status (PTS); and thermal taster status (TTS), a recently reported index of orosensory responsiveness. Taster status in PTS and/or TTS confers greater responsiveness to most orosensory stimuli. Gender, age, ethnicity, and fungiform papillae (FP) density were not associated with orosensory responsiveness to tastants, an astringent, and a flavour. Unlike PROP responsiveness, FP density was not associated with TTS. Both PROP responsiveness and TTS were associated with increased responsiveness to orosensory stimuli, including temperature and astringency. For PROP, this association did not hold when stimuli were presented at cold or warm temperatures, which are ecologically valid since most foods and beverages are not consumed at ambient temperature. Thermal tasters (TTs), who perceive 'phantom' taste sensations with lingual thermal stimulation, were more responsive to stimuli at both temperatures than thermal non-tasters (TnTs). While PTS, TTS, and gender affected self-reported liking and consumption of some alcoholic beverages, gender associated with the greatest number of beverage types and consumption parameters, with males generally liking and consuming alcoholic beverages more than females. Age and gender were the best predictors of alcoholic beverage liking and consumption. As expected, liking of bitter and fatty foods and cream was inversely related to PROP responsiveness. TTS did not associate with body mass index or waist circumference, and contrary to previous studies, neither did PROP responsiveness. Taken together, TnTs' greater liking of cooked fruits and vegetables and high alcohol, and astringent alcoholic beverages than TTs suggests differences between TTS groups may be driven by perceived temperature and texture. Neither an interaction between PTS and TTS nor a TTS effect on PROP responsiveness was observed, suggesting these two indices of individual variation exert their influences on orosensory perception independently.

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I know nothing except the fact of my ignorance.

-Socrates

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LIST OF ACRONYMS AND ABBREVIATIONS

Acronym/Abbreviation	Expansion
alum	aluminum sulfate
AMY	α-amylase
ANOVA	analysis of variance
ASTM	American Society for Testing and Materials
AUC	total area under the time-intensity curve
AVI	alanine-valine-isoleucine *TASR38* allele
BMI	body mass index
C	Cooked
CN	cranial nerve
d/m	total drinks consumed per month
DAng	angle (°) of sensation from IMax to the last recorded value
DArea	decrease area – area under the descending portion of the curve from Imax to the last recorded value
DUR	total duration (s) that the sensation is rated
F	alcohol consumption frequency
FP	fungiform papillae
gLMS	generalized labeled magnitude scale
GPCR	G-protein coupled receptor
gVAS	generalized visual analogue scale
HPLC	high performance liquid chromatography
HRP	histatin-rich protein
IAng	angle (°) of sensation increase from start to Imax
IArea	increase area - area under the ascending portion of the curve from start to Imax
IDelay	initial delay – time (s) to first response
IInt	initial intensity – first intensity response
IMax	maximum intensity
ISO	International Standards Organization
Lf	Lactoferrin
LMS	labeled magnitude scale
MG	mucin-glycoprotein
MR	Mechanoreceptor
MSG	monosodium glutamate
PAV	proline-alanine valine *TASR38* allele
pMT or MT	PROP medium-taster
pNT or NT	PROP non-taster
PROP	6-n-propylthiouracil
PRP	proline-rich protein

pST or ST	PROP super-taster
PTC	phenylthiocarbamide
PTS	PROP taster status
Q	beverages consumed per drinking occasion
R	Raw
RA	rapidly adapting
SA	slowly adapting
SD	standard deviation
SEM	standard error of the mean
SFR	salivary flow rate
TI	time-intensity
TMax	time (s) to maximum intensity
TnT	thermal non-taster
TPRM5	transient receptor potential melastatin 5 channel
TRC	taste receptor cell
TRP	transient receptor potential
TT	thermal taster
TTS	thermal taster status
WC	waist circumference
$\eta 2$	Eta squared

CHAPTER 1: INTRODUCTION

The flavour of food, which includes its taste, aroma, and mouthfeel, may be the most important predictor of dietary choice (Rozin, 1982; Glanz et al., 1998; Duffy, 2007). Given the importance of diet in wellness and disease (Hu et al., 2001; Kaput, 2004; Low and Tai, 2007), an understanding of the relationships between flavour components, sources of individual variation in the perception of flavour, and the consumptive outcomes of those factors is both timely and necessary.

The oral tactile sensation of astringency plays a significant role in the sensory experience elicited by a diverse range of foods and beverages (reviewed in Joslyn and Goldstein, 1964; Martin-Tanguy et al., 1977; Karchesy and Hemingway, 1986; Barahona et al., 1997; Gawel, 1998; Scharbert et al., 2004; Al Mahfuz et al., 2004; Ozawa et al., 1987). Numerous and potent health-promoting benefits of some astringent compounds (polyphenolics) found in a range of fruits and vegetables and the processed consumables derived from them have been described (Horiba et al., 1991; Renaud, and de Lorgeril, 1992; Fitzpatrick et al., 1993; Imai and Nakachi, 1995; Clifford et al., 1996; Hollman et al., 1997; Fuhrman et al., 2001; Landrault et al., 2003; Auger et al., 2005; Bargallo et al., 2006).

Gustatory sensations (i.e., tastes) receive great attention in the literature, however, far fewer studies are focused on the sensation of astringency. While the importance of astringency eliciting compounds in the human diet is generally accepted and appreciated, the influence of biological sources of individual variation, which include gender, ethnicity, age, physiology, and genetic variation, on the perception of astringency has not been extensively examined. Additionally, studies that have investigated these questions have returned equivocal results. For example, some studies have reported that salivary flow rate (SFR), a physiological measure, is greater in those individuals that perceive astringency with greater intensity (Peleg et al., 1999), while others have reported the opposite (Fisher et al., 1994; Ishikawa and Noble, 1995), and still others have reported no relationship (Guinard et al., 1998).

Since Fox's initial discovery almost 80 years ago that some individuals perceive phenylthiocarbamide (PTC) as bitter while others do not (Fox, 1931), responsiveness to

PTC, and subsequently the safer and odourless compound 6-n-propylthiouracil (PROP), has been used as a marker of genetic variability in the perception of taste. Bartoshuk and colleagues found that responsiveness to PROP follows a tri-modal distribution and demonstrated with suprathreshold scaling methods that individuals could be categorized into three PROP taster status (PTS) groups: super-tasters (pSTs) are able to perceive the bitterness of PROP at very low concentrations, medium-tasters (pMTs) perceive it at moderate concentrations, and non-tasters (pNTs) are minimally or non-responsive even at high concentrations (Bartoshuk et al., 1994; Bartoshuk et al., 2003; Bartoshuk et al., 2004). Molecular data indicate that the *TAS2R38* gene encodes two major forms of the PROP receptor, PAV and AVI, which results in three genotypes: PAV homozygotes, PAV/AVI heterozygotes, and AVI homozygotes (Kim et al., 2003). PTS groups were thought to represent *TAS2R38* genotypes where pSTs are PAV homozygotes, pMTs are heterozygotes, and pNTs are AVI homozygotes, however, recent work suggests that there is overlap between genotypes in their PROP responsiveness, and that other genes are likely to play a role in responsiveness to PROP (Duffy et al., 2004a; Hayes et al., 2008).

PROP responsiveness is also associated with increased responsiveness to other orosensory stimuli, or super-tasting (i.e., elevated response to taste, retronasal, somatosensory, and chemesthetic stimuli; Hayes et al., 2008). Prototypical tastants (i.e., salt, sugar, acid), including other bitterants, are perceived with greater intensity by pSTs than pNTs (Tepper et al. 2009; Bartoshuk et al.,1998; Prescott et al., 2001; Hayes et al., 2008). Irritation from ethanol is also perceived with greater intensity by those that perceive PROP to be more bitter (Bartoshuk et al., 1993; Bartoshuk et al., 1994; Prescott & Swain-Campbell, 2000; Duffy et al., 2004b). Evidence also suggests that pSTs are more responsive to some stimuli presented retronasally, and have lower orthonasal thresholds for some stimuli than pNTs and pMTs (Pickering et al., 2006; Yackinous & Guinard, 2001). The influence of PROP responsiveness and PTS on the perception of astringency has returned conflicting results. Contrary to findings that PTS does not affect astringency (Ishikawa and Noble, 1995; Sowalsky and Noble, 1998), Pickering et al. (2004) demonstrated that pSTs and pMTs found the astringency of red wines significantly more intense than pNTs, but in the same study reported that the

intensity of alum astringency was not associated with PTS.

Fungiform papillae (FP), which house taste receptor cells appear to be the physiological correlate underlying the phenomenon of PROP super-tasting, with pSTs having a higher FP density than pMT or pNT (Bartoshuk et al., 1994; Tepper and Nurse, 1997, Essick et al., 2003). Spatial summation occurs on the tongue (Smith, 1971), and evidence suggests that in individuals that perceive bitterness from PROP the increased innervation density that occurs with higher FP density is analogous to the increase in intensity when a larger area is stimulated (Delwiche et al., 2001). FP not only house taste receptors, but they are also surrounded and inhabited by fibers of the trigeminal nerve, which convey tactile information (Farbman & Hellekant, 1978; Whitehead et al., 1985). Indeed, pSTs demonstrate better lingual tactile acuity and rate tactile qualities of wine (i.e., particulate, smoothness, grippy/adhesive, and mouthcoat) higher than pNTs and (Essick et al., 2003; Pickering & Robert, 2006). Taken together, these data suggest FP density may provide a potential link between PROP responsiveness and astringency responsiveness.

Recently, thermal taste, a new marker of individual variation in oral sensation, was described (Cruz & Green, 2000). When a small area of the tongue is heated and/or cooled, thermal tasters (TTs), who constitute approximately 50% of the population sampled, perceive a phantom taste (Green & George, 2004). Thermal sweetness is most likely to occur on the tongue tip when it is re-warmed from an initial cooling period, thermal saltiness is sometimes reported upon cooling the same area, and thermal sourness is elicited in some individuals when the lateral edge of the tongue is cooled (Cruz & Green, 2000). Not only do TTs perceive a taste sensation from thermal stimuli, they also demonstrate super-tasting characteristics rating salt, citric acid, quinine, PROP, monosodium glutamate, and sucrose, as significantly more intense than thermal non-tasters (TnTs) (Green & George, 2004). Evidence from *Trpm5* (transient receptor potential melastatin 5) channel knockout mice indicates that TRPM5, a TRP superfamily cation channel with a role in the transduction of umami, sweet and bitter tastes (Zhang et al., 2003), plays a role in thermal taste (Talavera et al., 2005), suggesting that this source of individual variation is under genetic control.

TRPM5 is Ca^{2+}-activated and temperature-sensitive, with inward currents increasing significantly between 15°C and 35°C (and declining between 35°C and 40°C) due to a temperature-dependent shift of the channel's activation curve (Talavera et al., 2005; Talavera et al., 2007). The TRPM5 channel is suggested to act as coincidence detector of taste and temperature stimuli (Talavera et al., 2005). It is interestingly to note that, although it has yet to be determined whether basal levels of intracellular Ca^{2+} and heat alone can lead to TRPM5 activation (Talavera et al., 2005), the heating regimen used to elicit sweet thermal taste in humans is a ramp from 15°C to 40°C at approximately 1°C/s (Cruz & Green, 2000). Further, augmentation of the chorda tympani response to sweet stimuli observed when it is delivered in conjunction with elevated temperatures (35°C) is abolished in *Trpm5* knockout mice (Talavera et al., 2005). Indeed, many ion channels involved in taste transduction are postulated to act as coincidence detectors of temperature and depolarizing taste stimuli (Talavera et al., 2005; Talavera et al., 2007); however, for most channels, this possibility has not been examined *in vivo* or *in vitro*.

Interestingly, and somewhat surprisingly given the many conventions around the serving temperatures of foods (e.g., white wine should be served chilled), the effect of temperature on the perception of orosensory stimuli has not received much attention in the literature. A positive relationship has been described for the perceived intensity of astringency and temperature; however, this data comes from the study of a complex beverage, and the small but significant decreases described were coincident with decreases in viscosity, a parameter known to affect astringency perception (Smith et al., 1996). To-date, the effect of temperature on the perceived intensity of a simple astringent solution has not been described, nor has the effect of temperature on the perception of orosensory stimuli been examined using dynamic measures, such as time-intensity (TI) methodology.

Flavour perception is associated with liking and consumption of food and alcoholic beverages (Glanz et al., 1998; Lanier et al., 2005). While the relationship between PTS and PROP responsiveness and liking and consumption has been studied, the association between TTS and food behaviour has not been examined. PROP is hypothesized to mediate food choice via taste perception and food preference, and to

contribute to disease via diet, and/or through the development of obesity, which is associated with increased disease risk (Tepper, 2004; Duffy et al., 2004c). The increased responsiveness of pSTs to tastant solutions appears to translate into increased responsiveness to those same taste qualities in food. Hedonic responses also vary with perceived PROP intensity and PTS. For example, pNTs are more likely to be sweet 'likers', those whose hedonic responses increase with increasing sweetness, while pSTs are more likely to be sweet 'dislikers', those whose hedonic responses decreased with increasing sweetness (Looy & Weingarten, 1992; Yeomans, et al., 2007). It is hypothesized that pNTs' greater liking of high-fat foods leads them to consume more high-fat foods, which, over time, could lead to increased weight gain and obesity-related disease (Duffy, 2007; Tepper & Ullrich, 2002). Indeed, higher BMI and body fatness have been found in pNTs compared to pSTs (Goldstein et al., 2005). The increased acceptance of alcohol-related sensations and the higher consumption of alcoholic beverages by pNTs could also contribute to an increased risk of disease and illness (Duffy et al., 2004a; 2004c; Intranuovo et al. 1998; Guinard et al. 1996). The associations of PROP with food liking and consumption, and BMI are moderated by gender (Duffy et al. 1999; Tepper et al. 1998; Keller et al. 2009), dietary restraint (Tepper et al., 2002; Tepper et al., 2008), and food adventurousness, a measure of neophobia (Ullrich et al., 2004). Again, these parameters have not been examined in TTS, a potential genetic marker of individual variation that, like PROP responsiveness associates with orosensory perception.

Given the many and various foods and beverages that contain astringent compounds and their purported importance to health and disease states, the perception of astringency and factors that modulate that perception are of interest to numerous areas of research and many industries. The following dissertation provides a detailed examination of astringency, and examines the influence of PTS, TTS, and other sources of individual variation on the perceived intensity of a range of orosensory stimuli including astringency. The relationship between alcoholic beverage liking and consumption and food liking and markers of individual variation in oral sensation are also investigated. Further, the influence of temperature and markers of individual

variation on the time-course of perceived intensity of orosensory stimuli was examined using TI methodology.

Structure of Dissertation

Each chapter of this dissertation was written as a manuscript for publication. For this reason, some material may be redundant between chapters.

Chapter 2

Chapter 2 is a comprehensive review of compounds eliciting the sensation of astringency, the perception of oral astringency, and the chemical and physiological mechanisms responsible for astringency. Chapter 2 also provides an overview of the mechanisms involved in taste transduction. Further, Chapter 2 discusses known modulators of astringency and their proposed mechanisms. Chapter 2 was published in its entirety in *Critical Reviews in Food Science and Nutrition* in 2008 (Bajec and Pickering, 2008a).

Chapter 3

Chapter 3 examines the influence of sources of individual variation on the perception of orosensory stimuli, including astringent, metallic, sweet, sour, bitter, and salty stimuli. The main goal of this study was to investigate the influence of PTS and TTS on the perception of orosensory stimuli, and to examine a possible relationship between PTS and TTS. Gender, age, and fungiform papillae density were also examined as sources of variation. Further, ethnicity, and smoker status were included as factors. Chapter 3 was published in *Physiology and Behavior* in 2008 (Bajec & Pickering, 2008b).

Chapter 4

Chapter 4 examines the influence of TTS and PTS on perception of stimuli at two temperatures using TI methodology. In real-life consumption of foods and beverages, temperature varies quite dramatically. Consequently, the perception of orosensory stimuli changes over the course of consumption. To determine whether the results of Chapter 3 were robust and ecologically valid with regard to temperatures encountered during normal eating behaviours, the perceived intensity of orosensory stimuli presented at different temperatures was measured from the onset of the sensation to its extinction. Astringent, sweet, sour, and bitter stimuli were presented at 5°C and at

35°C, which allowed the effect of temperature on the perception of orosensory stimuli to be examined. It was hypothesized that if TTs' taste transduction pathways are more sensitive to temperature than TnTs, as suggested by their response to thermal stimuli, they may present differences in TI parameters of taste when it is delivered in conjunction with thermal stimuli. Further, if all or some channels involved in taste transduction are taste/temperature coincidence detectors, as has been suggested, and are more sensitive to temperature in TTs, then TTs' responses to the tastant that chemically elicits the taste they perceive upon thermal stimulation (i.e., sweetness on warming, bitter on cooling, etc.) may differ at different temperatures. Chapter 4 was prepared for publication in the journal *Chemosensory Perception.*

Chapter 5

Chapter 5 aims to elucidate the influence of TTS, PTS, fungiform papillae density, age, gender, and the perceived intensity of orosensory stimuli on alcoholic beverage liking and consumption. ANOVA was used to examine differences between groups, while correlations were used to determine relationships between continuous variables. Multiple regression was used to predict liking and consumption of alcoholic beverages from the sources of variation. Further, the association between liking and consumption was examined for each beverage. Chapter 5 was prepared for submission to the journal *Alcohol.*

Chapter 6

Chapter 6 investigates the influence of TTS and PTS on food liking. Subjects rated their liking of an extensive list of food items using a 7-point scale. Food items were grouped according to Food Groups, PTS and TTS Correlation Groups, and a novel grouping, Orosensory Groups. The Food Groups categorization grouped food items by food type. For PTS and TTS Correlation Groups food items were categorized according to their association with either PTS or TTS. Foods items that had a readily identifiable orosensory trait were grouped into Orosensory Groups. Chapter 6 was published in *Food Quality and Preference* in 2010 (Bajec & Pickering, 2010).

Chapter 7

Chapter 7 provides a summary and conclusions for the previous chapters, and suggestions for future work.

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CHAPTER 2: ASTRINGENCY: MECHANISMS AND PERCEPTION

Introduction

Astringency plays a significant role in the sensory experience elicited by a diverse range of foods and beverages (Table 1), including wine (Gawel, 1998), tea (Scharbert et al., 2004), soymilk (Al Mahfuz et al., 2004), coffee (Morales, 1989), fruits (Joslyn and Goldstein, 1964; Ozawa et al., 1987), nuts (Karchesy and Hemingway, 1986), and legumes (Martin-Tanguy et al., 1977; Barahona et al., 1997). Numerous and potent health-promoting benefits of some astringent compounds (polyphenols) found in a range of fruits and vegetables and the processed consumables derived from them, such as tea (Horiba et al., 1991; Imai and Nakachi, 1995; Hollman et al., 1997), red wine (Renaud, and de Lorgeril, 1992; Fitzpatrick et al., 1993; Clifford et al., 1996; Bargallo et al., 2006), and most recently, polyphenol-enriched white wines (Fuhrman et al., 2001; Landrault et al., 2003; Auger et al., 2005), have also been demonstrated. The importance of astringent compounds in the primate diet has been confirmed by the natural feeding behavior of rhesus monkeys (*Macaca mulatta*), who choose their food based on phenolic content as opposed to total protein or non-structural carbohydrate content (Marks et al., 1988).

While G-protein coupled receptors (GPCRs) and ion channels are generally - although not universally - accepted (Herness and Gilbertson, 1999; Bradbury 2004) as the molecular basis for sweet, bitter and umami tastes (Naim et al., 1994; Hoon et al., 1999; Adler et al., 2000; Chandrashekar et al., 2000; Matsunami et al., 2000; Li et al., 2002; Nelson et al., 2001; Nelson et al., 2002; Ozeck et al., 2004), and salty and sour tastes (Heck et al., 1984; Tennissen and McCutcheon, 1996; Kinnamon et al., 1988; Waldmann and Champigny, 1997; Ugawa et al., 2003), the molecular and physiological mechanisms underlying astringency have not been definitively elucidated (Bakker, 1998). At the perceptual level, it is far from clear whether astringency is best regarded as a single perceptual phenomenon or as a composite term encompassing a number of subtle tactile sensations (astringent 'sub-qualities'). Widely differing opinions exist on the current state of knowledge concerning astringency, with some groups claiming that the physical nature of astringency is well understood (Lyman and Green, 1990) while

Table 1. Common tannin-containing plants used as foodstuffs, forage crops, livestock feeds, beverages, and herbal preparations. Adapted from Haslam and Lilley (1988).

Proanthocyanidins (Condensed tannins)		**Galloyl & hexahydroxydiphenoyl esters (Hydrolysable tannins)**	
Common name	**Species name**	**Common name**	**Species name**
Apple	*Malus* sp.	Blackberry, Dewberry, Raspberry	*Rubus* sp.
Persimmon	*Diospyros kaki*	Walnut	*Juglans* sp.
Grape	*Vitis vinifera*	Strawberry	*Fragaria* sp.
Strawberry	*Fragaria* sp.	Carob pods	*Ceratonia siliqua*
Blackberry, Dewberry, Raspberry	*Rubus* sp.	Rose hip flower	*Rosa* sp.
		Pomegranate	*Punica granatum*
		Acorn	*Quercus* sp.
Plum, Cherry	*Prunus* sp.	Tea	*Camellia sinensis*
Bilberry, Cranberry	*Vaccinium* sp.	Uva-ursi	*Arctostaphylos uva ursi*
Gooseberry, Black & red currant	*Ribes* sp.	Paeony root	*Paeonia* sp.
		Geranium root – geranii Herba	*Geranium* sp.
Quince	*Cydonia* sp., *Chaenomeles chinensis*	Smoke-tree	*Cotinus coggyria*
		Cloves-flower buds	*Eugenia caryophyllata*
Cocoa bean	*Theobroma cacao*	Witch hazel	*Hamamelis* sp.
Kola nut	*Cola acuminata*	Kinimizuhiki	*Agrimonia japonica*
Pear	*Pyrus* sp.	Ohebi-ichigo	*Potentilla*

			kleiniana
Hawthorn	*Crataegus* sp.	Kibushi-leaves & fruit	*Stachyrus praecox*
Rose hip	*Rosa* sp.	Rhubarb	*Rhei rhizoma*
Chinese gooseberry	*Actinidia chinensis*	*Casuarina*	*Casuarina stricta*
Yam	*Dioscorea alata*	Sweet gum leaves	*Liquidambar* sp.
Sorghum	*Sorghum* sp.	Pistacio	*Pistacia vera, P. chinensis*
Barley	*Hordeum vulgare*	Guava	*Psidium guava*
Sainfoin	*Onobrychis viccifolia*	Nupharis rhizoma	*Nuphar japonicum*
Herbaceous legumes	*Lotus* sp., *Trifolium* sp. *Coronilla varia, Lespedeza cuneata, Lathyrus pratense*	*Bergenia* leaves & roots	*Bergenia crassifolia, B. cordifolia, B. purpurascens*
Heather	*Calluna vulgaris*	*Acacia* leaves	*Acacia milotica*
Wattle	*Acacia* sp.	Myricaceae bark	*Myrica rubra*
Rhubarb	*Rhei rhizoma*	Persimmon	*Diospyros kaki*
Polygonum multiflorum root	*Polygonum multflorum*	Myricaceae bark	*Myrica rubra*
Myricaceae bark	*Myrica rubra*		

others maintain the opposite (Bakker, 1998; Iiyama et al., 1995; Courregelongue et al., 1999). For instance, while some research strongly suggests that the sensation of astringency is a tactile phenomenon (Breslin et al., 1993), initiated by the binding and precipitation of proteins by polyphenols (Kallithraka et al., 1998), evidence has also been presented supporting the speculation of Aristotle and Galen that astringency is a gustatory sensation (Bartoshuk, 1978). This paper reviews the literature on this debate and others concerning the underlying mechanism(s) responsible for astringency, and provides an overview of the research concerned with elucidating the physical, physiological and psychological factors that mediate its perception.

Astringency and Astringents Defined

The American Society for Testing and Materials (ASTM) defines astringency as "the complex of sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins" (ASTM, 2004). Lee and Lawless (1991) presented evidence suggesting that the tactile attributes of drying, puckering, roughing, and overall astringency may not be totally interchangeable. Since the time-courses of "dry", "rough", and astringent sensations are well matched to the time-course of astringency, and the time-courses of puckering, bitterness, and sourness differ subtly from astringency when elicited by compounds commonly accepted as astringent (tannic acid, aluminum sulfate (alum) and tartaric acid), these authors suggest that there may be multiple sub-qualities to astringency. Green (1993) suggests this result implies that pucker, sourness and bitterness are not essential to the sensation of astringency. Lee and Lawless (1991), however, explicitly recommend that future studies of astringency address, and account for the possibility of multiple sub-qualities. Following their own advice, Lawless et al. (1994) developed a lexicon for the description of alum, gallic acid, catechin, citric acid and their mixtures consisting of the terms drying, roughing, puckery, and astringent. Lawless and Corrigan (1994) provided a graphic interpretation of the relationship between astringent sub-qualities, and their relationship with sourness, and they note that some of what are considered sub-qualities of astringency, such as drying, might be better described as concomitant reactions. Echoing Lawless' call for semantic agreement in the use of astringency descriptors,

Kiehlhorn and Thorngate (1999) in their examination flavan-3-ols conclude "...a refinement of the language used to describe oral sensations is necessitated."

To further refine discussions and descriptions of perceived astringency, Gawel et al. (2000) used descriptive data and clustering techniques to develop a hierarchical lexicon ("mouth-feel wheel") to assist in identifying and classifying a wide range of oral sensations elicited by red wine, which included 33 terms or sub-qualities to define astringency (Fig. 1). The majority of the publications considered in this review include, at a minimum, dryness, roughing and puckering in their definitions of astringency (Gawel, 1998; Simon et al., 1992; Ishikawa and Noble, 1995; Jöbstl et al., 2004), although some substitute constricting for puckering (Breslin et al., 1991), or employ either dryness (Lyman and Green, 1990) or puckering (Bate-Smith, 1954), and others explicitly provide no working definition of astringency (Fischer et al., 1994).

Astringent compounds

Medically, an astringent compound is considered "a drug that causes cells to shrink by precipitating proteins from their surfaces" (CMD, 2007). The current chemical and pharmacological definition of an astringent compound as one that binds and precipitates proteins has not deviated from its Latin root *ad stringere*, meaning "to bind" (Joslyn and Goldstein, 1964). Astringent compounds can be found in countless products ranging from skin cream to pickles. As described by Joslyn and Goldstein (1964), there are four groups of "true" (i.e., perceptually astringent and capable of reacting with proteins) astringent compounds: salts of multivalent metallic cations (particularly aluminum salts), dehydrating agents (ethanol and acetone), mineral and organic acids, and polyphenols.

Tannins, so named because of their use of in the process of tanning animal hides (Hergert, 1989), have long been considered an important component of some plants' defense mechanisms (Feeny, 1976) where they can act as either a digestive inhibitor or a toxin, depending on the tannin-type and its consumer (Robbins et al., 1991). Whether defense the primary function of tannins in plants has not yet been determined (Beart et al., 1985; Haslam, 1988). Tannins, also commonly referred to as vegetable polyphenols or polyphenols, are the primary source of astringency in foods and beverages reported

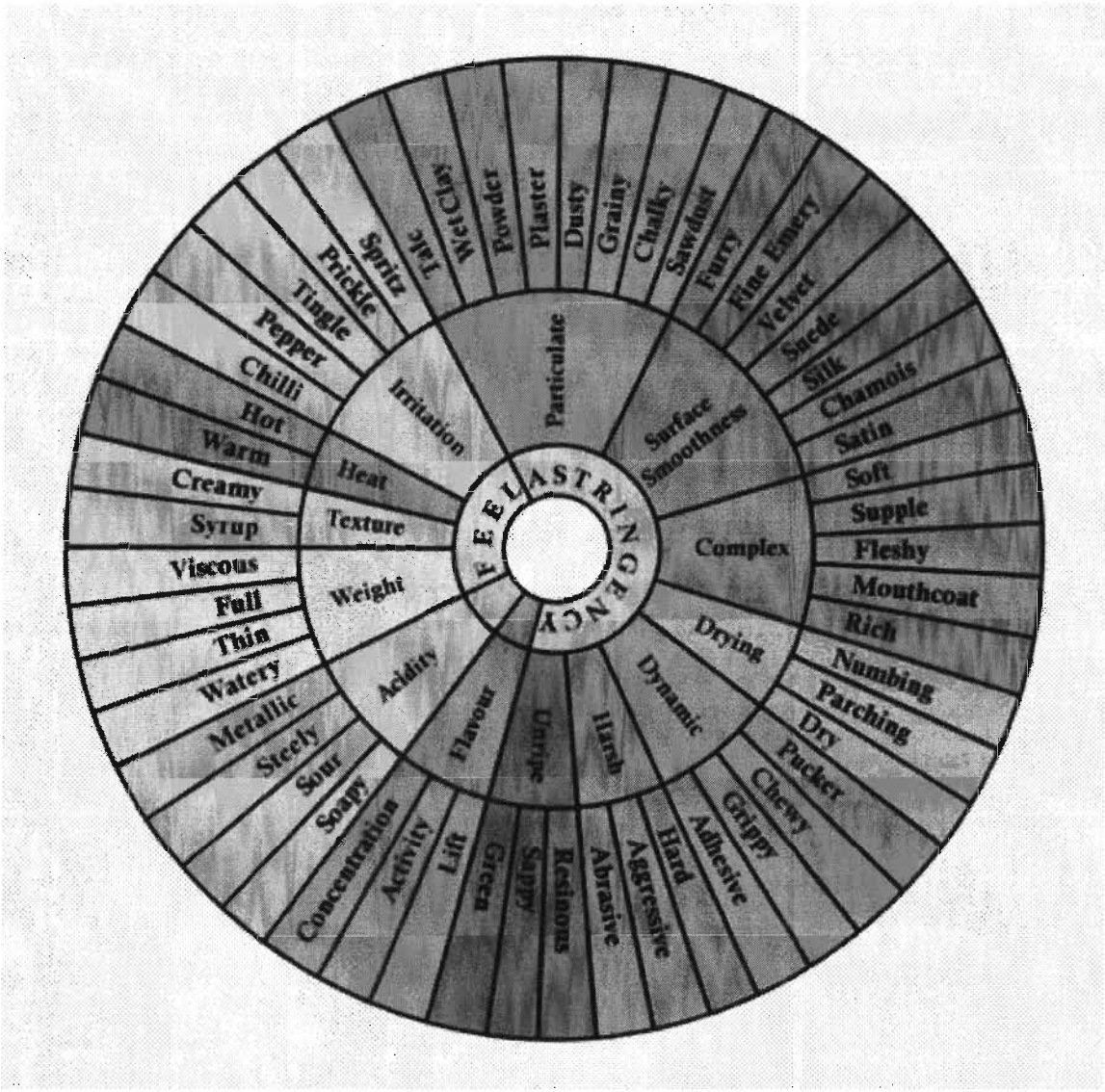

Figure 1. Red wine mouth-feel wheel.

Reproduced from Richard Gawel, A. Oberholster and I. Leigh Francis (2000); *Australian Journal of Grape and Wine Research* Vol 6(3), 203-207 (with permission from the Australian Society of Viticulture and Oenology).

to be astringent (Joslyn and Goldstein, 1964; Courregelongue et al., 1999; Bate-Smith, 1954; Arnold et al., 1980). Tannins are categorized as either condensed or hydrolysable, which are composed of proanthocyanidins, and galloyl and hexahydroxydiphenoyl esters, respectively (Haslam and Lilley, 1988; Bennick, 2002). Tannins have a number of anti-nutritional characteristics including: iron absorption inhibition (Disler et al., 1975), esophageal (Warner and Azen, 1988) and hepatic cancers (Korpassy, 1961), developmental inhibition and anomalies (Featherston and Rogler, 1975; Elkin et al., 1978), and irreversible complexation of digestive enzymes and dietary proteins (Robbins et al., 1991; Ahmed et al., 1991). However, the review of tannins and human health by Chung et al. (1998) suggests that tannins in moderation are responsible for a number of positive physiological effects. Tannins have also been shown to confer antibacterial, antimicrobial (Scalbert, 1991), anticarcinogenic (Das et al., 1989; Athar, et al., 1989), antioxidant (Teissedre et al., 1996), and neuroprotective effects (Sun et al., 1999; Sun et al., 2002; Simonyi et al., 2002).

Traditionally, astringent polyphenols have been defined as having molecular weights of being between 500 and 3000 Da (Bakker, 1998; Lesschaeve and Noble, 2005); however, smaller compounds, including 5-O-caffeoylquinic acid, and flavan-3-ol monomers, dimers and trimers, can also elicit astringency (Naish et al., 1993; Peleg et al., 1999). While simple phenols bind proteins weakly (Haslam and Lilley, 1988), it is generally accepted that the greater the polymerization and molecular weight of an astringent compound, the greater its ability to precipitate proteins (Bate-Smith, 1973), and its perceived intensity (Arnold et al., 1980; Peleg et al., 1999). Contrary to this experimental finding, the astringency of ripening fruit and aging red wine is reported to decrease with increased polyphenol polymerization (Ozawa et al., 1987; Waterhouse, 2002). In wine this decrease in astringency may be due to the occurrence of a number of chemical processes, not only polymerization (Vidal et al., 2002). Ozawa et al. (1987) suggests that the decrease in ripening fruits is not due to changes in polyphenols, but rather to changes in other molecules (e.g. pectin) that inhibit the interaction between polyphenols and mucosal proteins. Taira et al. (1997) provided in vitro evidence of this, as the perceived astringency of persimmons was reduced by the addition of pectin, or by a pectin pre-rinse.

Along with sourness, organic (i.e., acetic, fumeric, quinic, adipic, lactic, malic, tartaric, and citric) (Martin and Pangborn, 1971; Hyde and Pangborn, 1978; Rubico and McDaniel, 1992; Hartwig and McDaniel, 1995; Sowalsky and Noble, 1998), and inorganic acids (hydrochloric and phosphoric) (Rubico and McDaniel, 1992; Hartwig and McDaniel, 1995; Sowalsky and Noble, 1998; Corrigan and Lawless, 1995) can induce sensations of astringency. For organic acids, an inverse, pH-dependent relationship exists between acidity and perceived astringency (Hartwig and McDaniel, 1995; Sowalsky and Noble, 1998; Lawless et al., 1996). Aluminum sulfate (alum) is also an established astringent (Joslyn and Goldstein, 1964; Ward, 1882), and a component of a number of commonly used items, including: antiperspirants, toothpastes, cosmetics, soaps, eardrops, and topical astringents and styptics.

Ingestion of zinc also induces a primary sensation of astringency (Keast, 2003; Lim and Lawless, 2005), while for iron, copper, and the minerals magnesium and calcium the sensation of astringency is a secondary characteristic (Lim and Lawless, 2005; Lawless et al., 2004). Considering the recent trend toward mineral fortification of foods and beverages (Mitchell, 2004) and the need to make these products palatable (Hurrell, 2002), further research on the astringent properties of minerals is particularly pertinent (Lim and Lawless, 2005).

Mechanisms of Astringency Perception

Oral Physiology

The mammalian tongue houses three types of gustatory papillae: circumvallate, foliate, and fungiform. Polarized, neuroepithelial taste receptor cells (TRCs) in clusters of 50 to 150 are organized into taste buds in each papilla (Beidler, 1978). The apical surface of the taste bud is exposed to the oral cavity through the taste pore, where the microvilli of TRCs make contact with saliva and tastants (Akabas, 1990). Interestingly, TRCs are not static receptor structures. As first demonstrated in the rat, TRCs undergo a progression from basal cells, which are the precursor cell population, through differentiation and death that ranges from 2 days to 3 weeks (Beidler and Smallman, 1965; Hamamichi et al., 2006). TRCs themselves are not neurons; they synapse onto the

primary gustatory fibers of the nerves that innervate them, with each gustatory fiber contacting multiple TRCs in multiple taste buds (Scott, 2005).

Besides taste receptors, the oral cavity also houses mechanoreceptors (MRs), which appear to be of equal, if not greater, importance for astringency perception (Weiffenbach, 1993; Trulsson and Essick, 1997). Unlike TRCs, MRs are neurons that are classified according to the size and character of their receptive field (Kaas, 2004); type I MRs have small and distinct receptive fields, while type II have large, diffuse receptive fields (Jacobs et al., 2002). MRs are further classified depending on whether they are rapidly adapting (RA) or slowly adapting (SA) receptors; RA receptors respond during the dynamic phase of stimulus application and SA receptors respond to both dynamic and static force applications (Jacobs et al., 2002). The MRs in the oral cavity include: Ruffini endings, Merkel cells, Meissner cells (lamellated corpuscles), and free nerve endings (Capra, 1995; Watanabe, 2004). The distribution of MR types varies with oral cavity location. For example, recording from the infraorbital nerve, Johansson et al. (1988) found that about one-third of the MRs at the transitional zone of the upper lip were of the SA I (slow adapting, type I), while Trulsson and Essick (1997), recording from the lingual nerve, found that two-thirds of the MRs stimulated in the lingual mucosa were RA. Trulsson and Essick (1997) suggest that mucosal regions that are deformed during normal functioning (e.g., lips) have a greater proportion of SA afferents, while regions that are mainly used for explorative and manipulative behaviors (e.g., tongue) contain a proportionately greater number of RA fibers. While oral MRs appear to function like those of the skin, they have smaller receptive fields and lower activation thresholds (Trulsson and Essick, 1997).

The facial nerve (cranial nerve (CN) VII), the glossopharyngeal nerve (CN IX), and the trigeminal nerve (CN V) innervate the oral cavity (Matthews, 2001). Taste buds in the posterior one-third of the tongue receive innervation from the glossopharyngeal nerve, while those in the anterior two-thirds receive innervation from the chorda tympani branch of the facial nerve (Ottoson, 1983). Specifically, chorda tympani fibers innervate fungiform papillae and the facial nerve fibers serve the foliate and circumvallate papillae (Scott, 2005). Divisions of the mandibular branch of the trigeminal nerve, namely the lingual nerves, also project to the anterior portion of the

tongue providing somatosensory innervation (Trulsson and Essick, 1997; Biedenbach and Chan, 1971). Not only do these fibers innervate the epithelia surrounding the taste buds, in rodents they have also been shown to enter fungiform papillae (Farbman and Hellekant, 1978; Whitehead et al., 1985). The mandibular and infraorbital nerves provide innervation to the mucus membranes of the lower lip and cheeks, and upper lip and cheeks, respectively (Johansson et al., 1988). The territory innervated by the trigeminal nerve extends to include the teeth, periodontium, and the bulk of both the soft and hard palates (Capra, 1995). All of these nerves - infraorbital nerve, chorda tympani, lingual nerve, glossopharyngeal nerve - contain afferent mechanoreceptive fibers (Trulsson and Essick, 1997; Johansson et al., 1988; Biedenbach and Chan, 1971; Oakley, 1985; Herness, 1988).

The salivary glands are under collaborative parasympathetic (acetylcholine) and sympathetic (noradrenalin) control via the efferent (secreto-motor) fibers of the facial and glossopharyngeal nerves (Garrett, 1967; Garrett and Kidd, 1993). The majority of saliva is secreted by the parotid, submaxillary/submandibular, and sublingual exocrine glands (Dawes and Wood, 1973). At rest, the submaxillary glands contribute 69%, the parotid 26%, and the sublingual contributes only 5% to the total secretions of these salivary gland pairs (Schneyer and Levin, 1955). With stimulation, the parotid gland increases its contribution to the total secretions of these gland pairs by 8%, while the submandibular gland and the sublingual gland decrease their contributions by 6% and 2.2%, respectively (Schneyer and Levin, 1955). It is through these glands that salivary proteins and enzymes are secreted into the oral cavity, where they provide lubrication and initiate the process of digestion (Young and Schneyer, 1981).

Salivary Proteins

Total salivary protein composition varies greatly between individuals (Jenzano et al., 1986; Lu and Bennick, 1998; Asikyan, 2005). Values between 1.135 mg/ml and 3.8 mg/ml have been reported in total protein concentration of whole stimulated saliva (Lenander-Lumikari et al., 1998; Martins et al., 2006), and in unstimulated saliva values between 0.9 mg/ml and 7 mg/ml have been reported (Nederfors et al., 1994; Agha-Hosseini et al. 2006). Recent research into the salivary proteome has identified 437

proteins in salvia (Xie et al., 2005), but only those implicated in the sensation of astringency will be discussed here, namely proline-rich proteins (PRPs), histatins (HRPs), α-amylase, lactoferrin (Lf), and mucins (Lu and Bennick, 1998; Yan and Bennick, 1995; Gambuti et al., 2006; Condelli et al., 2006; de Freitas and Mateus, 2001).

The proline-rich proteins (PRPs) are one of the main protein families secreted from the parotid and submandibular glands. The members of this 20-protein family are most commonly noted in considerations of astringency as they interact with and precipitate polyphenols (Hagerman and Butler, 1981). PRPs are characterized by their highly repetitive structure of approximately 19 residues of proline, glycine and glutamine repeated 5-15 times, which alone accounts for 70-80% of the total amino acid content of PRPs (Kauffman and Keller, 1979). The three types of PRPs, basic, acidic, and glycosylated account for 23%, 30%, and 17%, respectively, of the total protein in parotid saliva, with PRPs overall comprising 70% of salivary proteins (Kauffman and Keller, 1979; Bennick, 1982). Although all the PRPs' functions have yet to be fully elucidated, acidic PRPs may have a role in calcium homeostasis and bacterial binding (Bennick et al., 1981; Amano et al., 1996), and glycosylated PRPs provide lubrication (Hatton et al., 1985) and prevent bacterial agglutination (Bergey et al., 1986). The 11, 6-9 kDa basic PRPs have demonstrated anti-viral activity, and a high affinity for binding tannins (Lu and Bennick, 1998; Hagerman and Butler, 1981; Mehansho et al., 1983; Kauffman et al., 1991).

Treatment of rats and mice with the β-adrenergic receptor agonist isoproterenol causes hypertrophy of the parotid and submandibular glands, and an increase in the production of PRPs (Muenzer et al., 1979a). Interestingly, feeding rats and mice a sorghum diet high in tannins has the same results as isoproterenol injection, but the effects are restricted to the parotid gland (Mehansho et al., 1983; 1985), indicating that PRP levels are modulated by the concentration of tannins in these rodent's diet. In parallel with the PRP increase, rats fed a high-tannin diet gained weight, implying that the increase in PRP secretion has a positive nutritional effect on the animal (Mehansho et al., 1983). The results of Mehansho et al. (1983) and Asquith et al. (1985) suggest

that the increased PRP secretion in mice and rats fed high-tannin diets results from β-adrenergic receptor activation.

While hamsters also responded to isoproterenol treatment with increased levels of PRPs, their response to a high-tannin diet was quite different than that observed in rats and mice (Mehansho et al., 1987). Hamsters did not respond to a high-tannin diet with a compensatory increase in PRP levels, rather they displayed severely retarded growth and/or death. After six months on a high-tannin diet hamsters were the same size as they were at 3 days old, but when switched to a low-tannin diet they grew at close to the same rate as younger animals on a normal diet (Mehansho et al., 1987). The variation in tannin-handling capabilities between rats, mice, and hamsters did not result from differences in either their β-adrenergic receptor complement or their adenylate cyclase activity (Mehansho et al., 1987). These results, along with those of Robbins et al. (1991) suggest that similar animals with seemingly homologous proteins cannot be expected to react in the same way to dietary tannin.

It has been suggested that other mammalian omnivores and herbivores produce PRPs constitutively, at a concentration that reflects the approximate level of polyphenols in their diets (Luck et al., 1994; McArthur et al., 1995). This suggestion is substantiated by the fact that mammalian herbivores, whose diets do not naturally include tannin-containing foods, do not produce tannin-binding salivary proteins (Austin et al., 1989). High performance liquid chromatography (HPLC) analysis of saliva following the ingestion of highly astringent wine suggests that humans might also modulate PRP levels based on polyphenol consumption, as, for some subjects, late-eluting peaks observed post-ingestion increased in area (Kallithraka et al., 1998). The results of Asikyan (2005) appear to confirm this finding; while some salivary proteins decrease in concentration following ingestion of astringent red wine, others, in the molecular weight range of the PRPs, are reported to increase.

Twelve low molecular weight histatins, or histidine-rich proteins (HRPs) have been isolated (Troxler et al., 1990; Oppenheim et al., 1988), and are only found in saliva (Sabatini et al., 1989). HRP1, 3, and 5, found in parotid and submandibular secretions, are the predominant members of the family, accounting for 80% of all HRPs present in saliva (Lamkin & Oppenheim, 1993). Interestingly, a lower concentration of HRPs is

found in whole salvia than in pure parotid and submandibular glandular secretions due to their degradation in whole saliva (Baum et al., 1976). Aside from their roles in the maintenance and protection of tooth enamel, HRPs participate in non-immune oral defense through their potent anti-microbial action (Lamkin and Oppenheim, 1993; Oppenheim et al., 1986; 2007).

α-amylase catalyzes the hydrolysis of α(1,4)glycosidic bonds of polysaccharides, and is found in organisms ranging from insects to humans. Salivary α-amylase is composed of two families; the glycosylated A family, and the non-glycosylated B family. The A family comprise isoenzymes 1, 3 and 5, and family B comprise isoenzymes 2, 4 and 6 (Oppenheim et al., 2007; Keller et al., 1971). α-amylase secretion from the parotid gland increases with stimulation by tastes (Froehlich et al., 1987), and its bacterial-binding capacity suggests it may contribute to bacterial clearance, and it has been detected in dental plaque (Scannapieco et al., 1993).

Lactoferrin (Lf), a relatively minor component of saliva (Dodds et al., 2005), is a member of the transferrin family of non-heme iron-binding proteins (Levay and Viljoen, 1995) that is present in all salivary glands (Reitamo et al 1980). While multiple isoforms of Lf exist, the iron-binding Lf is an 80 kDa, single chain protein, whose tertiary structure consists of two ferric- and glycan-binding globular lobes (Levay and Viljoen, 1995; Lonnerdal et al., 1995). Lf acts as an antibacterial via nutritional immunity by making iron unavailable as a food source and thus starving the bacteria (Humphrey and Williamcon, 2001). Lf also has direct bacteriostatic effects; for some bacteria these effects depend on Lf being iron-free, while for others the effects depend on Lf being bound by iron (Aguilera et al., 1998; Fine & Furgang, 2002).

Mucin-glycoproteins, or mucins, are principally responsible for the viscoelastic properties of all mucosal secretions, including saliva (Schenkels et al., 1995; Tabak, 1990). The submandibular and sublingual glands, along with some of the minor salivary glands, secrete the two main salivary mucins, MG1 and MG2 (Tabak, 1995). MG1 is a large multi-subunit superstructure, with a high-carbohydrate content and hydrophobic pockets, whose molecular weight is in excess of 1000 kDa (Tabak, 1990; Loomis et al., 1987). MG2 is a low-molecular weight (200-250 kDa) single polypeptide chain, enriched in threonine, serine, proline, and alanine (Loomis et al., 1987). As might be

expected, MG1 provides better lubrication than MG2 (Aguirre et al., 1989), and binds tightly to teeth contributing to the protective enamel pellicle (Humphrey and Williamson, 2001). Interestingly, MG2 is easily displaced from enamel, but has demonstrated important functions in the aggregation and clearance of oral microorganisms (Schenkels et al., 1995).

Polyphenol-Protein Binding

Some have suggested that the binding of polyphenols by proteins is a defense mechanism that inhibits harmful tannins before they become bioavailable and affect the gastrointestinal tract (Lu and Bennick, 1998; Hagerman and Butler, 1981; Mehansho et al., 1987; McArthur et al., 1995; Mehansho et al., 1987; 1995). Another plausible explanation is tannin detection. Based on the particle sizes resulting from mastication, and the unlikelihood that mastication would release all of the tannins located in intracellular vacuoles thus leaving some tannins to traverse the gastrointestinal system unbound by proteins, it is also theorized that the interaction of tannins with salivary proteins and the sensation of astringency are part of a mechanism for the detection of potentially harmful astringent compounds (Prinz and Lucas, 2000).

Regardless of whether it is a defense or a detection mechanism, the protein-binding ability of polyphenols is well documented, and has been demonstrated with a variety of proteins besides salivary PRPs, including: casein (Jöbstl et al., 2004; Luck et al., 1994), gelatin (Hagerman and Bulter, 1981; Oh et al., 1980; Yokotsuka and Singleton, 1995; Siebert et al., 1996; Edelmann and Lendl, 2002), bovine serum albumin (BSA) (Hagerman and Butler 1981; 1980; 1980) haemoglobin (Bate-Smith, 1973), pectin (Hayashi et al., 2005), and HRPs (Yan and Bennick, 1995; Naurato et al., 1999). Most recently, data has been presented indicating that mucins (Monteleone et al., 2004; Condelli et al., 2006), Lf, and α-amylase are also capable of polyphenol-binding (Gambuti et al., 2006; de Freitas and Mateus, 2001; 2001), and that, along with the PRPs and HRPs, these proteins are involved with the sensation of astringency.

PRPs appear to have a higher affinity for condensed tannins than for hydrolysable tannins, and for polymers over monomers (Yokotsuka and Singleton, 1995; Baxter et al., 1997). Similarly, larger PRPs have a greater affinity for tannins than

smaller PRPs or peptide fragments (Hagerman and Butler, 1981; Charlton et al., 2002). The greater affinity of larger, polymerized polyphenols for proteins, and vice versa, has been attributed to the multidentate nature of polyphenols, which allows a single polyphenol to bind multiple residues of the protein (Jöbstl et al., 2004; Baxter et al., 1997; Charlton et al., 2002). In the case of hydrolysable tannins, the affinity of tannin-protein binding is directly related to the degree of galloylation, as pentagalloylglucose binds proteins with greater affinity than monogalloylglucose (Baxter et al., 1997; Charlton et al., 2002; Kawamoto et al., 1995). The effect of galloylation on binding affinity reaches a plateau with the pentagalloylated molecules, as the affinity of hepta- and octagalloylglucose for PRPs is of the same order as tetra- and pentagalloylglucose (McManus et al., 1985; Bacon and Rhodes, 2000).

Protein-tannin complexes have been described as both soluble and insoluble, and recent data suggests that complex solubility is dependent on a number of variables. Using BSA and a condensed tannin, Hagerman and Robbins (1987) demonstrated that, under optimal protein:polyphenol ratios and pH conditions, protein-polyphenol complexes are insoluble. However, in the presence of excess protein, the protein-polyphenol complexes that form are soluble as there is not enough tannin to sufficiently crosslink proteins and form aggregates (Hagerman and Robbins, 1987). Luck et al. (1994) confirmed these results using gelatin and a hydrolysable tannin, but using salivary PRPs they were unable to resolublize the polyphenol-protein complex, regardless of how much protein was added. These findings suggest that the stability of polyphenol-protein complexes depends not only on the environmental conditions of the reaction (Hagerman and Robbins, 1987; Kawamoto and Nakatsubo, 1997), but also on the types of polyphenol and protein used.

The first studies of polyphenol-protein binding used condensed tannins in their examinations, and the results suggested mainly hydrogen bonding between the hydroxyl groups of the polyphenols and the carbonyl groups of the proteins (Hagerman and Butler, 1981; 1980; 1980). Subsequent studies have confirmed that for condensed tannins, hydrogen bonding is the driving force of the interaction (Oh et al., 1980; Hagerman et al., 1998; Simon et al., 2003), but in some cases, it appears that hydrophobic interactions may be the basis for the complexation of tannins with protein

(Jöbstl et al., 2004; Luck et al., 1994; Baxter et al., 1997; Charlton et al., 2002; Hagerman et al., 1998). Hagerman et al. (1998) suggest that polyphenol polarity is the main predictor of the type of association that will occur between polyphenols and proteins (i.e., hydrogen bond vs. hydrophobic interaction), with polar polyphenols forming hydrogen bonds and nonpolar polyphenols forming hydrophobic interactions.

Charlton et al. (2002) put forth a 3-stage model of the binding and precipitation of PRPs by polyphenols. Jöbstl et al. (2004) have confirmed and expanded the model (Fig. 2). In step 1, the binding of multiple multidentate polyphenols to several sites on the protein causes the previously randomly coiled protein to coil around the polyphenol, making the protein more compact. In the second stage, the polyphenol fractions of the protein-phenol complexes cross-link forming polyphenol bridges and creating protein dimers, and finally (step 3), the dimers aggregate to form large complexes and precipitate. The initial polyphenol-protein interaction results from the binding of the hydrophobic face of the polyphenol's aromatic ring with the pyrrolidine ring of the protein's proline residues (Charlton et al., 2002). Jöbstl et al. (2004) suggest that this 3-stage model is consistent with the time-course of astringency; however this assertion has yet to be confirmed.

Besides their antibacterial and antifungal roles, HRPs have also been identified as polyphenol-binding proteins, which suggests a possible role for them in the perception of astringency (Yan and Bennick, 1995; Naurato et al., 1999). While HRP1, HRP3, HRP5, and HRP7 are capable of binding tannins, the amount of tannin bound appears to vary with the type of tannin used (i.e., condensed vs. hydrolysable) (Naurato et al., 1999). Yan and Bennick (1995) demonstrated that HRP5 was more efficient than PRP1 at precipitating tannic acid, a hydrolysable tannin, as well as a condensed tannin at a pH of 7.4, but PRP1 was a more effective precipitator of both polyphenol preparations at a pH of 3.0.

Although it does so with lower affinity than the PRPs, α-amylase readily binds both tannin types, which inhibits its activity (de Freitas and Mateus, 2001; Kandra et al., 2004; Zajacz et al., 2006). The α-amylase-tannin interaction is reversible, leaving α-amylase activity intact after its release from the tannin, and is inhibited by both HRP5 and an acidic PRP (Yan and Bennick, 1995; Oh et al., 1980). Similarly, mucins have

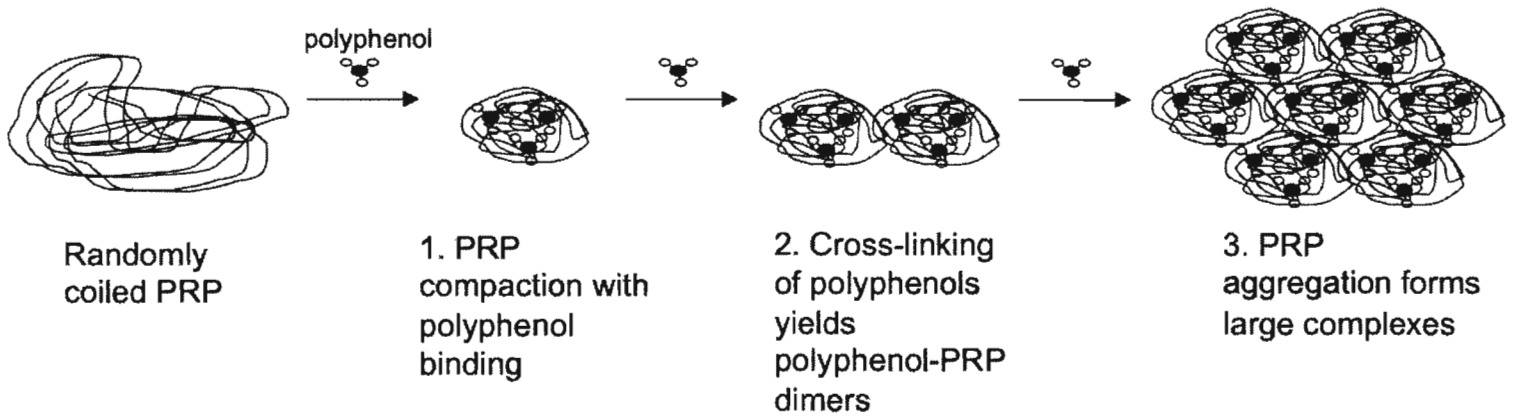

Figure 2. Proposed mechanism for PRP-polyphenol binding and subsequent protein aggregation and complex formation. Adapted from Jobstl *et al.* (2004).

been shown to bind polyphenols (Gambuti et al., 2006; Monteleone et al., 2004; Conedlli et al., 2006), and, along with α-amylase, Lf and two glycosylated PRPs, decrease in saliva following ingestion of astringent wine (Gambuti et al., 2006).

Together, these results strongly suggest that tannin-binding is a redundant function of salivary proteins, which confirms its physiological importance (Bennick, 2002).

Astringency as a Tactile Sensation

In 1954, Bate-Smith first suggested that astringency is a feeling, not a taste, and since then the postulated tactile nature of astringency has been accepted as a paradigm. Joslyn and Goldstein (1964) furthered the tactile theory of astringency by asserting that "(T)he precipitation of tissue proteins is accompanied by the shrinkage of tissue due to a loss of water and a decrease in the permeability of this tissue to water and solutes". They further postulated that astringency might be the result of a constriction or closure of the salivary ducts, or inhibition of the salivary gland causing a decrease in available saliva. Given that salivary flow rate increases in response to an astringent compound in complex and model solutions (Lyman and Green, 1990; Fischer et al., 1994; Hyde and Pangborn, 1978), this is no longer tenable.

Evidence supporting a reduction in salivary PRPs following ingestion of an astringent solution came from Kallithraka et al. (1998), who attributed the decrease in tentative PRPs following wine intake to their precipitation resulting from their complexation with phenols. While some have suggested that the precipitation of salivary and epithelial proteins leads to a constriction of the oral epithelium (Joslyn and Goldstein, 1964; Lyman and Green, 1990), or that astringent substances change the oral epithelium causing it to feel rough (Jellinek, 1985), a 2-stage model where the polyphenol-protein interaction precedes the binding of the complex to the epithelial proteins has also been put forth (Guinard et al., 1986a) and expanded to include recent polyphenol-polyphenol binding data (Jöbstl et al., 2004). The current 'lubrication' theory of astringency asserts that after astringent compounds strip the oral cavity of mucosal and epithelial proteins that confer lubrication, the increased friction between the surfaces of the oral cavity stimulates mechanoreceptors (Lyman and Green, 1990).

The interaction of proteins and polyphenols in solution results in the development of a haze or cloudiness (Monteleone et al., 2004; de Freitas and Mateus, 2001), which can be observed in complex matrices such as beer and wine (Siebert et al., 1996), and simple mixtures of saliva and tannic acid (Horne et al., 2002). A negative correlation between astringency ratings and haze developing capacity was observed when individuals' saliva was mixed with tannic acid (Horne et al., 2002), suggesting that a higher level of salivary proteins available to bind polyphenols results in a decrease in perceived astringency. Conversely, the results of Kallithraka et al. (2001) suggest that protein binding and precipitation are not directly related to the perception of astringency, as the time course of chemical astringency (i.e., protein binding) was not correlated to its perception. This result corroborates the findings of Guinard et al. (1998) who found no correlation between the perception of astringency and salivary protein composition.

The only direct physiological data indicating that astringency is a tactile sensation mediated by non-gustatory mechanisms comes from research presented by Breslin et al. (1993) and Lim and Lawless (2005). These studies demonstrate that aluminum sulfate (Breslin et al., 1993) and copper sulfate (Lim and Lawless, 2005) elicit the sensation of astringency when applied to the area between the gum and the upper lip, an area of the mouth generally accepted to be devoid of taste receptors (Jones, 1954). Green (1993) suggests that the MRs responsible for astringency may be RA afferents that have been identified in the chorda tympani and lingual nerve (Trulsson and Essick, 1997;Biedenbach and Chan, 1971).

Besides this direct evidence, the theory of astringency as a tactile sensation is based on characteristic differences between astringency and the five accepted gustatory sensations. One line of argument concerns adaptation, "...the disappearance of taste impressions under continuous stimulation" (Moskowitz, 1978), likely resulting from receptor-dependent mechanisms, such as desensitization (O'Mahony, 1986; Bohm et al., 1997; Meyerhof et al., 2005). Adaptation of each of the five tastes has been demonstrated (Abrahams et al., 1937; Krakauer and Dallenbach, 1937; McBurney and Lucas, 1966; Meiselman, 1968; O'Mahony 1989; Gent and McBurney, 1978). It has been suggested that, since the perceived intensity of an astringent stimuli increases with repeated ingestion (Lyman and Green, 1990; Courregelongue et al., 1999; Guinard et

al., 1986) and other tastes decrease in intensity with repeated ingestion, astringency cannot be a gustatory sensation (Green, 1993). In contradiction to this line of reasoning, the perceived intensity of bitterness, which is an accepted gustatory sensation, has also been shown to increase with sustained or repeated ingestions (Lyman and Green, 1990; McNulty and Moskowitz, 1974; Guinard et al., 1986). While Lyman and Green (1990) note of their results that "(T)he lack of adaptation may have been due in part to the intermittent (once per minute) pattern of stimulation, which may have allowed at least partial recovery from adaptation", they do not indicate how the recovery from adaptation might result in an increase in intensity with repeated sampling.

The ability of augmented oral lubrication to decrease the astringency intensity of polyphenols and alum has also been put forth as evidence that astringency is a tactile phenomenon (Lyman and Green, 1990; Courregelongue et al., 1999; Breslin et al., 1993; Smith et al., 1996; Smith and Noble, 1998; Peleg and Noble, 1999; Brannan et al., 2001). A number of compounds have been employed to increase the viscosity of astringent solutions, such as carboxymethylcellulose (Courregelongue et al., 1999; Smith et al., 1996; Smith and Noble, 1998; Peleg and Noble, 1999), actual and artificial saliva (Breslin et al., 1993), temperature (Peleg and Noble, 1999), and sucrose (Lyman and Green, 1990; Courregelongue et al., 1999; Breslin et al., 1993; Ishikawa and Noble, 1995; Smith et al., 1996). As discussed below, the effect of sucrose on astringency is likely not solely due to its function as a thickener. If friction between oral surfaces leads to the activation of MRs and the perception of astringency, one might expect that a universal lubricant like oil would reduce perceived astringency, but this does not always appear to be the case. While a mixture of corn oil and xanthan gum very effectively decreased the perceived astringency of alum (Breslin et al., 1993; Brannan et al., 2001), corn oil alone had no effect on the astringency elicited by soymilk (Courregelongue et al., 1999). The variation in these results may be due to differences in the astringent mechanisms of soymilk and alum; however, they may also suggest that the ability of viscous agents to bind tannins may be of greater importance in mediating astringency than their capacity as simple lubricants. Matrix viscosity has been shown to affect the intensity of accepted gustatory sensations and complex flavours (Moskowitz and Arabie, 1970; Christensen, 1980; Malkki et al., 1993; Walker and Prescott, 2000;

Hollowood et al., 2002), but not bitterness (Smith et al., 1996). These results can be taken to suggest that viscosity, by some as yet unknown mechanism, is a general modulator of taste and flavour rather than as evidence that astringency is a tactile phenomenon.

Astringency as a Taste

The results of Kawamura et al. (1969) directly demonstrate that tannic acid interacts with the oral epithelium, and provide evidence that tannic acid does not directly interact with MRs. They also show that, not only is astringency an unpalatable sensation in rats, tannic acid stimulates fibers in the glossopharyngeal nerve and the chorda tympani, but not the lingual nerve. Based on their findings that tannic, tartaric, and gallic acids elicit a rapid and reversible response in the chorda tympani, but not the lingual nerve, Schiffman et al. (1992) concluded that astringency is a taste sensation.

A direct conclusion regarding the gustatory nature of astringency is difficult to make based on the electrophysiological data of Kawamura et al. (1969) and Schiffman et al. (1992). While their results clearly indicate that the chorda tympani and glossopharyngeal nerve are responsive to astringent compounds, the basis for their conclusion that astringency is a taste may not be valid. Schiffman et al. (1992) describe the lingual nerve as responsive to tactile, thermal, and pain sensations, but this nerve is also responsive to chemical stimulation (Wang et al., 1993) and Schiffman et al. (1992) themselves demonstrate that it is responsive to some of the high-concentration, low-pH astringents presented. They conclude that since astringent compounds that stimulated the chorda tympani did not stimulate the lingual nerve, MRs cannot be involved in the perception of astringency. These studies clearly demonstrate that the collaborative innervation of the anterior two-thirds of the tongue by the chorda tympani and the lingual nerve confers the ability to respond to a wide array of chemical stimuli, but they fall short of providing definitive proof that astringency is a taste. It is interesting to note that while the tactile theory of astringency postulates that its tactile nature stems from the increased friction between oral surfaces after the loss of lubrication, both Kawamura et al. (1969) and Schiffman et al. (1992) claim that the unresponsiveness of the lingual

nerve, and thus MRs, to the direct application of astringent stimuli is evidence that astringency is not tactile.

The interaction of astringent compounds with ion channels has also been presented as evidence of the gustatory basis of astringency. Simon et al. (1992) further verified that astringents are capable of interacting with proteins through their demonstration that tannic acid and aluminum salts inhibit amiloride-sensitive Na^+ channels in preparations of isolated canine lingual epithelia. Similarly, the ability of tannic acid and catechin to alter the membrane potential of a lipid taste sensor has been presented as support for astringency being a gustatory sensation (Iiyama et al., 1995). These authors describe the effectiveness of catechin and tannic acid in the modulation of membrane potential, much like bitter and sour tastants do. The cellular effects of astringent substances have been found to culminate in cortical signaling (Critchley and Rolls, 1996). Single-cell recordings from neurons located in the orbitofrontal region, which includes the secondary taste cortex, clearly illustrate that a sub-population of neurons, the "tannic acid best" neurons, are responsive to as little as 1 mM tannic acid. Six of the 74 cells examined in two male behaving rhesus macaques responded to oral application of the astringent with a significant increase in frequency of action potential firing. An increased firing frequency for tannic acid best neurons was not observed when hydrochloric acid was applied, suggesting that these neurons are specific for tannic acid, or some component of it (Critchley and Rolls, 1996).

All of the studies discussed here conclude that astringency should be considered a distinct taste quality, like sweet, sour, salty, bitter, and umami (i.e., savory taste from meats, broths, etc.). Alternatively, we suggest that while these results indicate that astringent compounds are capable of interacting with cellular receptors, this does not discount the tactile theory of astringency. Taken together, the findings discussed above suggest that, for some astringent compounds, the sensation of astringency may be the result of both taste and tactile mechanisms working together.

Time-course and Measurement

In contrast to taste sensations, the perception of astringency builds slowly in intensity after ingestion and persists for a longer duration. Thus, for many

psychophysical studies and for product development research, time-intensity (TI) methods, where the perceived intensity of the sensation of interest is recorded for a specified duration, may be more appropriate for fully describing astringency responses (Noble 1994).

The perceived intensity of astringency increases linearly to a maximum at 13-15 seconds post-ingestion, regardless of the concentration of the astringent compound (Ishikawa and Noble, 1995; Guinard et al., 1986a). Using an experimental design considered comparable to normal wine consumption patterns, Guinard et al. (1986) demonstrated that the maximum intensity and the time to maximum (i.e., the time required to reach the maximum intensity) of perceived astringency is unchanged with repeated white wine ingestion (i.e., ingestion, swallowing, return to astringency intensity of zero, repeat), but the total duration of the astringent sensation increased with repeated ingestion. When wine was ingested repeatedly with only a 20- or 40-s interval between ingestions, a clear, significant increase in maximum intensity was observed (Guinard et al., 1986). Subsequently, Lyman and Green (1990) demonstrated that the intensity of a solution of tannic acid would continue to increase with repetitive intake (i.e., 10 ml in mouth for 10 seconds per minute) for 20 minutes.

According to Lee and Lawless (1991) the perception of astringency elicited by 750 mg/l tannic acid was not entirely extinguished six minutes post-expectoration. In contrast, the results of Guinard et al. (1986a), based on ingestion of white wine with 500 mg/l added tannic acid, indicate that astringency reaches intensity levels close to those pre-ingestion within 70 seconds after expectoration. Using 1000 mg/l tannic acid in water, Valentova et al. (2002) found that some residual astringency was perceivable at 100 seconds post-ingestion, and the results of Fischer et al. (1994) place extinction at approximately 120 seconds, both of which are in accord with Guinard et al. (1986).

Overall, these results reinforce the importance of experimental design and protocol when conducting sensory trials with astringents in order to account for the risk of carry-over and additive effects. One practical approach to help minimize these risks in psychophysical studies is the use of pectin mouth-rinse between samples (Colonna et al., 2004). Presumably, pectin is competing with PRPs for polyphenol-binding, and is

becoming increasingly employed to reduce carry-over and additive effects (Pickering and Roberts, 2006; Pickering et al., 2006).

The phenomenon of multiple astringency sub-qualities – at least at the perceptual level – discussed earlier raises concerns about the limitation of traditional one-dimensional visual-analog approaches (e.g., "rate the astringency intensity on the line scale") in capturing the full range of sensations experienced, particularly for products that elicit complex tactile sensations, such as red wine. The red wine "mouthfeel-wheel" (Gawel et al., 2000) is one recent innovation to assist enologists to more precisely and comprehensively describe and measure astringency (Fig. 1). While this multi-tiered lexicon includes some terms that appear more hedonic or composite in nature (e.g., "aggressive", "rich", "activity"), it is nonetheless proving valuable for describing the full range of astringent sensations elicited by red wines (Pickering and Robert, 2006; Geddes et al., 2001; DeMiglio et al., 2002; Francis et al., 2002; Vidal et al., 2003; DeMiglio, 2005).

Modulators of Astringency

pH

While acids are themselves astringent (Rubico and McDaniel, 1992; Hartwig and McDaniel, 1995; Sowalsky and Noble, 1998; Corrigan and Lawless, 1995), pH also affects the perceived intensity of astringents. Dealcoholised white wine with 1% ethanol, 1500 mg/l tannic acid, and a pH of 3.0 was found to be more astringent than the same wine with a pH of 3.6 (Fischer et al., 1994). Similar results have been obtained for red wines across an increasing pH series (pH 2.2, 2.4, 2.6 and 2.8) (DeMiglio, 2005), and when different acids are used (Kallithraka et al., 1997). Guinard et al. (1986) did not find the inverse relationship of pH and astringency in their high-phenol red wines, and suggest that this is because of the high starting astringency of the wines, which may have effectively precipitated the majority of salivary proteins negating a change in astringency by the addition of acids. In cranberry juice, a decrease in pH increased the perceived astringency, regardless of the temperature or viscosity of the juice (Peleg and Noble, 1999). Even simple aqueous solutions of phenolic compounds (i.e., grape seed tannins, tannic acid, catechin, gallic acid), and model solutions are affected by a

decrease in pH (Kallithraka et al., 1997; Guinard et al., 1986b; Peleg et al., 1998). The increased intensity of perceived astringency is likely due to the decrease in charged phenolate ions, which are unable to form hydrogen bonds with proteins, at low pH (Sowalsky and Noble, 1998; Guinard et al., 1986).

Interestingly, Peleg et al. (1998) found that the astringency of alum decreased with the addition of acid, and attribute this to the chelation of the aluminum ions in alum by acids, reducing its availability to interact with salivary proteins. These results indicate that alum and phenolic astringents cannot be used interchangeably in psychophysical studies (Peleg et al., 1998), and present the possibility that alum and tannic acid might elicit the sensation of astringency through different mechanisms.

Gustatory Sensations and Cross-modality Effects

Four basic gustatory sensations have been accepted as 'tastes', sweet, sour, bitter, and salty. A fifth taste, umami, while controversial is also generally accepted (Bradbury, 2004). Taste transduction occurs through either GPCRs (sweet, bitter, and umami) or ion channels (salty and sour), and while it is still unclear whether astringency should be classified as a taste or tactile sensation, taste qualities have been shown to physically and psychologically interact with and influence perception of astringency.

Lea and Arnold (1978) characterized bitterness and mouth dryness as 'twin sensations' since the two are often confused and almost all phenolic compounds that elicit astringency are also bitter. Other studies have also noted similarities between bitterness and astringency, and the ability to elicit both with the same compounds (Ishikawa and Noble, 1995; Peleg et al., 1999). However, polymeric tannins are more astringent than bitter while monomeric tannins are more bitter than astringent (Robichaud and Noble, 1990). Lee and Lawless (1991) dissected the sensations elicited by tannic acid and found that bitterness, along with sourness, a taste induced by acids, could be distinguished from astringency and separately rated, confirming discrete differences between them. The results of Bertino and Lawless (1993) further confirmed that panelists are able to distinguish between astringent qualities (e.g., dry, puckery, astringent) and gustatory sensations (e.g., bitter, sour, salty).

While evidence has been presented suggesting that all true tastes moderate astringency intensity (Brannan et al., 2001), most research in this regard has focused on sweetness (Lyman and Green, 1990; Courregelongue et al., 1999; Breslin et al., 1993; Ishikawa and Noble, 1995; Smith et al., 1996; Brannan et al., 2001; Speegle, 2002). The astringency of tannic acid (Lyman and Green, 1990) and red wine (Ishikawa and Noble, 1995) decrease in the presence of sucrose, possibly by interfering with the binding of tannins and salivary proteins. Equi-sweet solutions comprised of the non-nutritive sweetener aspartame have also been effective in reducing oral astringency (Speegle, 2002), but not to the same extent as sucrose (Lyman and Green, 1990). These authors suggest that the viscosity of the aspartame solution, which was markedly lower than that of the sucrose solution, was the reason it was not as effective as sucrose in reducing astringency. Using similar concentrations of aspartame Smith et al. (1996) presented contradictory evidence; they found that the sweetener did not affect the astringency of grape seed tannin solutions.

Variation in the Perception of Astringency

Salivary Flow Rate

An individual's salivary flow rate may affect their perception of astringency. Using white wine fortified with tannic acid, Fischer et al. (1994) demonstrated that subjects classified as having high and medium salivary flow rates perceived astringency sooner, for shorter duration, and with less intensity than those classified as having low salivary flow rates. The results of Ishikawa and Noble (1995), who divided participants into low (mean=1.92 g/min) and high (mean=3.73 g/min) salivary flow rates, corroborated those findings using a red wine matrix. Guinard et al. (1998) found no affect of salivary flow rate on the perception of astringency elicited by tannic acid fortified white wine. It has been postulated that more rapid re-lubrication (through a number of possible mechanisms) of the oral cavity occurs in individuals with higher flow rates, thus reducing the duration and intensity of the perceived astringency (Ishikawa and Noble, 1994; Noble, 1995). In contrast, Peleg et al. (1999) found that individuals with a high salivary flow rate perceived the intensity of astringent polyphenols as more intense than those with a low flow rate, while all other TI

parameters (i.e., time to maximum, total duration, time (from ingestion) to decay to 60% and 30% of maximum intensity) examined were unaffected by flow rate categorization. In their examination of organic acid astringency, Sowalsky and Noble (1998) found no difference in the astringency ratings of malic, lactic, tartaric, and citric acid between low, medium, and high salivary flow groups, which is in accordance with the results of Smith et al. (1996).

Foods and beverages, including alcoholic beverages, are typical sialogogues (Martin and Pangborn, 1971; Guinard et al., 1998; Guinard et al., 1997), and astringent compounds have also been shown to alter salivary flow rates (Hyde and Pangborn, 1978). Wine (Hyde and Pangborn, 1978), and wine augmented with tannic acid (Fischer et al., 1994; Guinard et al., 1998) have been shown to increase the rate at which saliva flows into the oral cavity, although Lyman and Green (1990) found no difference in salivary volume when ingestion of water was compared to tannic acid.

PROP Status

Sensitivity to 6-n-propylthiouracil (PROP) and phenylthiocarbamide (PTC) is genetically inherited and has been thought to follow an incomplete dominance mode of inheritance (Guo and Reed, 2001), allowing the classification of individuals into three groups reflecting their "PROP taster status" (PTS): non-tasters (NTs), medium-tasters (MTs) and super-tasters (STs; Bartoshuk, 2000). Recent molecular data indicates that there are three broad categories of PROP/PTC receptors encoded by the *hTAS2R38* gene; those that are sensitive to PROP/PTC, those with intermediate sensitivity, and those little or no sensitivity (Bufe et al., 2005). STs experience PROP as intensely bitter, MTs perceive PROP but less intensely than STs, and NTs cannot taste PROP or experience it as a very mild sensation. PTS serves as an index of general sensitivity to oral stimuli; the perceptual differences between PTS groups extend to other bitterants (Bartoshuk, 1979; Bartoshuk et al., 1988; Bartoshuk et al., 1993; Bartoshuk et al., 1996; Delwiche et al., 2001); salty compounds (Bartoshuk et al., 1998), sweet compounds (Gent and Bartoshuk, 1983), and substances that produce oral irritation/pain (Cunningham, 2000; Karrer and Bartoshuk, 1991) and tactile sensations (Duffy and Bartoshuk, 1996; Tepper and Nurse, 1997).

PTS is correlated with gender (Bartoshuk et al., 1994), food preferences (Drewnowski et al., 1997; 1999), alcoholism (Pelchat and Danowski, 1992; DiCarlo and Powers, 1998), and a number of diseases (Shepard and Gartler, 1960; Milunicova et al., 1969; Ahuja et al., 1977; Schlosberg and Baruch, 1992; Ali et al., 1994), demonstrating its importance in physiological function. The underlying basis for the perceptual differences between PTS groups appears itself to be physiological. Miller and Reedy (1990) found that individuals with a greater number of fungiform papillae have a greater number of taste pores, and rated PROP higher in intensity than those with low papillae and taste pore numbers. Later work (Reedy et al., 1993) confirmed that PROP STs have more fungiform papillae and taste pores than MTs and NTs. An interesting correlate has also been found between PTS and the ability to perceive tactile stimuli and differentiate tactile stimuli based on small differences; STs are better able to perceive small particles placed on the tongue than NTs (Tepper and Nurse, 1997; Chopra et al., 2002), recognize raised alphabet letters by tongue (Essick et al., 2003), and have a lower threshold for tactile stimulation (i.e., Von Frey filament stimulation) (Yackinous and Guinard, 2001). As the diameter of fungiform papillae is smaller and their density greater in STs, this, in conjunction with greater trigeminal innervation, might account for their greater tactile acuity.

While numerous studies have examined the interaction, a direct relationship between PROP status and astringency is not clear, as, to-date, results have been conflicting. Contrary to findings that PROP taster status does not affect astringency (Ishikawa and Noble, 1995; Sowalsky and Noble, 1998), Pickering et al. (2004) demonstrated that PROP STs and MTs found the astringency of red wines significantly more intense than NTs, but in the same study reported that the astringency intensity of alum was not PTS-dependent. Later, Pickering et al. (2006) report that STs perceive the intensity of alum with greater intensity than NTs, and suggest that the contradiction of this result with their previous findings may be attributable to the use of a substantially higher concentration in the former study. The lack of a PTS effect on perceived astringency in other studies may have been due to the technique used to categorize individuals and separate the groups, with more sensitive suprathreshold measures being used by Pickering et al. (2006). Imm and Lawless (1996) and Courregelongue et al.

(1999) also found that PTS impacted the perception of astringency, with NTs perceiving the astringency of alum and soymilk, respectively, higher than PROP tasters. Interestingly, Pickering and Robert (2006) found that STs rated the 'overall astringency' of red wines lower than NTs, but those same STs rated the textural sub-qualities of the wines higher (Table 2). They suggest that previous findings of higher astringency ratings by STs – where this was the only tactile attribute measured by the subjects - may be due, in part, to a dumping effect (Clark and Lawless, 1994). Given the greater tactile acuity of STs, presumably from their more densely packed fungiform papillae and/or greater trigeminal innervation, they may be better equipped to discriminate and rate finer qualities of astringency and other tactile sensations.

While the majority of studies indicate that PTS is a factor in an individual's perception of astringency, we recommend that future studies also incorporate a physiological measure (such as papillae density) to validate/assist with PTS classifications, as assignment of PTS based solely on PROP intensity ratings may lead to errors in categorization.

Conclusion

Taken together, the literature demonstrates that astringency is a complex, multifaceted sensation whose examination is complicated by a number of variables. While many studies have examined astringency, the lack of a clear, accepted definition that delineates the oral sensations it encompasses makes it difficult to effectively compare results. The potential interaction of astringency with basic tastes in many complex foods and beverages suggests that the physiological and psychological mechanisms underlying the perception of astringency should be further studied using simple, single-component stimuli. The use of single component stimuli would also give researchers greater ability to draw causal relationships between the stimuli used and the actual sensations perceived. Aroma is an interesting potential mediator of astringency that has recently received attention (Pickering et al., 2006); investigation into the possible role of olfaction may provide important insights into central and peripheral processes affecting astringency perception.

Table 2. Sensitivity to 6-n-propylthiouracil (PROP) and intensity ratings for tactile sensations elicited by red wine. Adapted from Pickering and Roberts (2006).

	PROP Taster Status*			
Sensation	**Non-tasters**	**Super-tasters**	**F-value**	**P(F)**
Particulate in mouth	3.86 ± 0.14	5.49 ± 0.25	37.091	0.000
Particulate after expectoration	4.90 ± 0.17	6.80 ± 0.20	69.575	0.000
Smoothness in mouth	5.64 ± 0.21	6.95 ± 0.25	20.415	0.000
Smoothness after expectoration	6.07 ± 0.21	7.70 ± 0.23	41.645	0.000
Grippy/adhesive	7.55 ± 0.23	9.21 ± 0.23	43.287	0.000
Mouthcoat	6.21 ± 0.20	7.92 ± 0.23	41.066	0.000
Overall astringency	6.89 ± 0.21	6.18 ± 0.26	6.847	0.009
Tingle/prickle	5.18 ± 0.18	6.04 ± 0.30	6.487	0.011
Viscosity	4.54 ± 0.19	3.98 ± 0.23	3.583	0.059
Heat/irritation	5.59 ± 0.17	6.36 ± 0.27	6.027	0.014
*for each PTS group, data shown are means values of 256 observations (16 wines x 8 subjects x 2 replicates ± std error				

The literature suggests that distinct astringent compounds may utilize different pathways in eliciting astringency and that both mechanical and chemical stimulation may contribute to the sensation. Given the time course of neuronal and perceptual processes reviewed here, a possible mechanism for astringency could involve the ingestion of astringent compounds, initially detected by the central nervous system, providing an assessment of the compounds and the current state of the oral and gastrointestinal environment to initiate the appropriate response through the peripheral nervous system (i.e., sequestering of astringent compounds by salivary protein secretion). Given the importance of astringent compounds to food preference (Kawamura et al., 1969; Marks et al., 1988; Glendinning, 1992) and health (e.g., Chung et al., 1998), elucidation of the pathways responsible, and greater clarity on how underlying physiology and genetics mediate the perception of astringency are necessary and timely.

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CHAPTER 3: THERMAL TASTE, PROP RESPONSIVENESS, AND PERCEPTION OF ORAL SENSATONS

Introduction

Food and beverage flavour strongly influences consumption [1], and consequently a range of health and disease outcomes. Thus, an understanding of the extent and sources of variation between individuals in flavour perception is of considerable interest to psychologists, nutritionists, and epidemiologists. Differences in the perception of taste and non-taste oral sensations result from a number of factors including, but not limited to, gender [2], age [3], ethnicity [4,5], salivary composition and salivary flow rate (SFR) [6], experience [7], and environment [8]. Arguably, however, the most important factor in the differences between individuals in perception of oral stimuli is genetic variation.

Since Fox's initial discovery over 70 years ago that some individuals perceive phenylthiocarbamide (PTC) as bitter while others do not [9], responsiveness to PTC, and subsequently to the odourless and non-carcinogenic 6-n-propylthiouracil (PROP), has been used as a marker of genetic variability in the perception of taste. While other genes are thought to be involved [10-12], molecular data indicate that the *TAS2R38* gene encodes two major forms of the PROP receptor, PAV and AVI; those individuals that carry two PAV alleles are very responsive to PROP, those with two AVI alleles are minimally or non-responsive, and those with one PAV allele and one AVI allele demonstrate intermediate responsiveness [13]. This finding is corroborated by years of psychophysical data that has identified three PROP taster phenotypes, typically expressed as PROP taster status (PTS) groupings [14]. Although PROP bitterness is a continuous measure of intensity, the somewhat artificial division of individuals into 3 PTS groups meets the constraints of analysis of variance (ANOVA). Here we adopt the nomenclature of Lim et al. [15] and Reed [16] for PTS, where, in order of descending responsiveness, the groups are: PROP super-tasters (pSTs), PROP medium-tasters (pMTs), and PROP non-tasters (pNTs). pSTs are able to perceive the bitterness of PROP at very low concentrations, pMTs perceive it at moderate concentrations, and pNTs are minimally or non-responsive even at high concentrations [2]. pSTs also perceive prototypical tastants (e.g., salt, sugar, acid), including other bitterants, with

greater intensity than pNTs [17-19], and are more sensitive to irritants [20-22], tactile stimuli [23-26] including astringency [27,28], and purportedly olfactory stimuli presented orthonasally and retronasally [24,27]. The relationship between PTS and astringency intensity has been debated in the literature, however Pickering et al. [28] suggest that the conflicting results may be due to the different PTS categorization methods and concentrations of astringents used.

The idea that PTS plays an important role in physiological and behavioural functions stems from its reported associations with gender [2], ethnicity [29], body mass index (BMI) [25,30], food preferences [31,32], alcoholism [33,34], and smoking [35]. Interestingly, some groups have also demonstrated a lack of association between PTS and BMI [24,36], and food preference [37] in adults, suggesting that such complex measures are influenced by a number of diverse variables besides taste intensity.

The physiological basis for the differences in oral perception between pSTs, pMTs, and pNTs appears to be fungiform papillae (FP) density and taste pore number, with PROP pSTs having a significantly greater number of FP and taste pores on the anterior surface of the tongue than pMTs and pNTs [2,25,38]. In individuals that perceive the bitterness of PROP, the increased innervation density that occurs with higher FP density is analogous to the increase in intensity when a larger area is stimulated, akin to spatial summation [39]. Stimulation of a small area of the anterior tongue in individuals with a greater FP density yields higher intensity ratings for NaCl, sucrose, and citric acid than for individuals with lower FP density [40-42]. Interestingly, however, FP density may not be associated with the perceived intensity of stimuli presented to the whole mouth [12,41]. Along with innervation from the glossopharyngeal nerve (posterior tongue) and chorda tympani (anterior tongue) [43], FP are also innervated by trigeminal fibers [44,45] that house mechanoreceptors [46], which suggests that, as with tastants, the link between perceived PROP intensity and tactile stimuli is FP, and concomitantly, innervation density [47].

Given the complexity of taste transduction and the physiological importance of the taste system [48], it is difficult to imagine that PTS alone is responsible for genetically-mediated differences in oral sensation. Recently, Green and coworkers identified a new marker of individual variation in oral sensation: thermal taste [49].

When a small area of the tongue is heated and/or cooled, thermal tasters (TTs), who constitute approximately 50% of the population sampled, perceive a phantom taste [50]. Thermal sweetness is most likely to occur on the tongue tip when it is re-warmed from an initial cooling period, thermal saltiness is sometimes reported upon cooling the same area, and thermal sourness is elicited in some individuals when the lateral edge of the tongue is cooled [49]. Evidence from *Trpm5* knockout mice strongly suggests that TRPM5, a TRP superfamily cation channel with a role in the transduction of umami, sweet and bitter tastes [51], plays a role in thermal taste [52], confirming that this source of individual variation is under genetic control. Not only do TTs perceive a taste sensation from thermal stimuli, they also rate salt, citric acid, quinine, PROP, and monosodium glutamate applied to the tongue tip, as well as whole-mouth rinses of sucrose, citric acid, and PROP, as significantly more intense than thermal non-tasters (TnTs) [50]. Vanillin presented orthonasally and retronasally, both with and without the addition of a tastant, was also rated as more intense by TTs, which may suggest that the heightened responsiveness to oral and olfactory stimuli results from differences in gustatory and olfactory brain region excitability [50]. Interestingly, however, ratings of burning, stinging and prickling produced by capsaicin and menthol did not differ between TTs and TnTs [53].

Thus, both PROP intensity and thermal taster status (TTS) appear to represent important and possibly independent proxies of general responsiveness to oral stimuli. However, in contrast to PROP, few studies exist on TTS and psychophysics, and no literature appears on its association with astringency, metallic flavour, or temperature perception. Additionally, the relative importance of PTS and TTS to general perception of oral stimuli and behaviour is not known. The present study examines the association between PTS and TTS and the basic tastes, an astringent, a flavour, FP density, and salivary flow rate in the same cohort of subjects. Further, we also investigate the relative contribution of PTS and TTS to perceived intensity of oral stimuli.

Methods

Subjects

126 subjects were recruited from the student, staff, and faculty populations of Brock University, and from the local community. Incentive was provided in the form of a monetary prize or credit toward a 1st year university Psychology course. Subjects consisted of 84 females and 42 males with a mean age of 31.1 years+/-11.5SD (range: 18 to 68). To establish ethnic origin, the Census Canada "Ethnic Origin User Guide" [54] was employed. Accordingly, and herein, the term Caucasian refers to those that reported 'White' as their ethnicity, and non-Caucasian refers to the group of subjects composed of 4 Southeast Asian, 3 South Asian, 4 Black, 4 Chinese, 2 Aboriginal, 1 Japanese, 1 Filipino, 1 Latin American, and 1 Iranian. One hundred and five subjects were Caucasian (32 males), and the remaining 21 were non-Caucasian (8 males). Twelve subjects reported that they smoked: 1 non-Caucasian female, 4 Caucasian females, and 7 Caucasian males. The Brock University Research Ethics Board approved all procedures, and written consent was obtained from all subjects.

Scale

Paper versions of the general Labeled Magnitude Scale (gLMS) were used to collect all psychophysical data [55,56]. The quasi-logarithmic gLMS, which is based on the original LMS [57,58], is anchored at its low and high extremes by the labels "no sensation" (0 mm) and "strongest imaginable sensation of any kind" (100 mm), respectively. Intermediate labels include "barely detectable" (1.4 mm), "weak" (6 mm), "moderate" (17 mm), "strong" (35 mm), and "very strong" (53 mm). Subjects received verbal and written instructions that the top of the scale represented the most intense sensation in any modality that they could ever imagine experiencing, and were told to think of experiences from a variety of different modalities to assist in understanding the general nature of the scale [55]. Paper questionnaires were employed to record demographic and other variables.

Procedure

Each subject attended 2 sessions of approximately 1 hour each. Tests and procedures were administered in the order in which they appear below and repeated in session 2 to obtain duplicate responses, with the exception of scale acclimation and FP density determination, which were performed only once (session 1). Tests were separated by a minimum interval of 5 minutes during which the subject completed questionnaires. All solutions were made with pure water (Millipore RiOs 16 Reverse Osmosis System, MA, USA), stored in the dark at 3-4 °C, and brought to room temperature (22 °C+/-2) well in advance of testing. Solutions were not kept for more than 7 days and were typically discarded within 5 days of preparation, with the exception of iron sulfate, which was used and discarded within 3 hours to avoid by-products of oxidation [59]. All samples were presented as 20 ml aliquots in ISO tasting glasses labeled with their contents.

Scale Acclimation

In order to familiarize subjects with the gLMS, and facilitate correct scale use, they were asked to rate the intensities of 5 remembered sensations: sourness of a lemon, pain from biting your tongue, coolness of an ice-cold beverage, burning sensation from eating a whole hot pepper, brightness of the sun when you are looking directly at it [60,61].

Prototypical Tastants, Astringents, and Metallic Stimuli

Aqueous samples of various oral stimuli were presented as exemplars of the different taste and non-taste oral qualities and to obtain ratings of perceived intensity. Stimulus concentrations for prototypical tastes were based on a review of the pertinent literature, followed by bench tests. Levels of aluminum sulfate (alum) and iron sulfate were based on Pickering et al. [27,62] and Lawless et al. [63], respectively. Subjects evaluated the intensity of low and high levels of astringent (0.73 mM and 14.6 mM alum; Sigma-Aldrich, MO, USA), salt (10.5 g/L NaCl; Windsor, QC, Canada), sweet (147.2 g/L sucrose; Lantic Sugar Ltd., QC, Canada), sour (4.47 mM tartaric acid; Carl Roth KG,

distributed by Atomergic Chemetals Corp., NY, USA), bitter (0.02 g/L quinine sulfate; Novopharm, ON, Canada), and metallic (0.3 mM and 3 mM iron (II) sulfate; J.T. Baker; NJ, USA) stimuli presented in random order. Subjects were instructed to put the entire sample volume, or as much as physically possible, in their mouths and rinse well for 5 s, being sure to cover all oral surfaces. Ten seconds post-expectoration, subjects were instructed to rate the maximum perceived intensity of the stimuli. A pectin rinse (5 g/L; Pomona's Universal Pectin, MA, USA) was taken after astringent samples to reduce possible carry-over effects [64], and filtered (Brita, ON, Canada) water rinses were performed after every sample and after pectin rinses. Both rinses were at room temperature (22°C+/-2). Filtered water was also available to subjects *ad libitum*. A minimum 1-minute rest was taken between samples, and subjects were instructed to take a longer break if needed. To aid in the identification of sensations, subjects were given additional verbal instruction regarding astringency; specifically, that it is a dry, puckering, constricting sensation often associated with red wine, tea, coffee, and unripe fruits [65].

Salivary Flow Rate (SFR)

Salivary flow rate (SFR) was determined as described in Ishikawa and Noble [66]. Subjects rinsed with 21 mM citric acid (Caledon Laboratories Ltd., ON, Canada) for 10 s, expectorated, then collected saliva in weighed polypropylene tubes (VWR, ON, Canada) for 1 minute. Samples were immediately weighed and SFR (g/min) calculated.

6-n-propylthiouracil (PROP)

A 0.32 mM solution of 6-n-propylthiouracil (PROP; MP Biomedicals; OH, USA) was prepared by dissolving PROP in water on a low heat stirring plate. Subjects rinsed with a 20 ml volume of the solution, or as much as physically possible, for 10 s, expectorated, and waited for the bitterness intensity to peak (on average 10-15 s) before providing a rating.

Fungiform Papillae (FP) Density

Fungiform papillae (FP) density was determined following the method of Shahbake et al. [67]. Subjects rinsed with distilled water before blue food colouring (Horton Spice Mills Ltd., ON, Canada) was applied to the tongue by the researcher using a piece of filter paper (Whatman's No. 1; Fisher, ON Canada). Following application of the dye, subjects rinsed with water again to remove excess dye. The tongue was then dried using a second piece of filter paper and a 1cm strip of filter paper was placed near the tongue tip as a scale. The subject rested their chin comfortably on a tissue-covered lab-jack (Fisher; ON, Canada) and extended their tongues as far as possible without contracting the tongue muscles. Images of the tongue were taken with a Canon Powershot S3 IS 6.0 megapixel camera in super macro mode mounted on a mini-tripod. Two, 15 cm, 100 W type A halogen bulbs (Home Hardware; ON, Canada) were mounted 30 cm above and 40 cm in front of either side the subjects head. Images were imported into and manipulated using Photoshop (CS2 9.0.2; Adobe; ON, Canada) on an iMac computer (Apple; CA, USA). Zoom, brightness, contrast, and colour balance functions in Photoshop were employed as needed to obtain the best image for analysis. A line was applied to the image using the line tool to demarcate the midline of the tongue. The filter paper scale was measured using the Photoshop measure tool and a 0.6 cm diameter circle was superimposed on the photo using the ellipse tool. All FP within a 0.6 cm diameter circle on each side of the anterior dorsal midline of the tongue were counted, averaged, and the FP density (FP/ cm^2) calculated.

Thermal Taste (TT)

In order to heat and cool small areas of the tongue, a thermode was built by the Brock University Electronics and Machine Shops after Cruz and Green [49], which consisted of a 64 mm^2 computer-controlled Peltier device with a thermocouple feedback attached to a toothbrush-sized water-circulated heat sink. For hygienic purposes, the thermode was covered with a fresh piece of plastic wrap (SC Johnson, WI, USA) for each subject, and rinsed with 95% ethanol (Rider Distillery Ltd.; LCBO, ON, Canada) between

subjects. Subjects extended their tongues while the thermode was gently, but firmly, applied by the researcher. Subjects were provided with a single sheet with 6 separate gLMS scales labeled 'temperature', 'sweet', 'salty', 'sour', 'bitter', and 'other'. 'Temperature' and 'other' scales were provided to avoid dumping effects [68], but allow for examination of perceived temperature intensities, and capture any other oral sensations that might be experienced during thermal stimulation. Subjects were instructed to rate the intensity of all oral sensations, including temperature that they perceived in each trial. Three locations on the edge of the tongue were stimulated discretely and in order: the most anterior tip, and approximately 1 cm to the right and then the left of the midline. The temperature ramp employed for all trials was approximately 1°C/s. Prior to each thermal taste session, a base line trial was performed where the thermode was applied to the tongue tip at body temperature (37°C) for 10 s and the subject practiced reporting perceived temperature and any oral sensations. Warming trials started at 35°C, cooled to 15°C, and re-warmed to 40°C where the temperature was held for 1 s. The start temperature for cooling trials was 35°C, followed by cooling to 5°C where the temperature was held for 10 s. Warming trials preceded cooling trials at each location to avoid possible adaptation from the intense, sustained cold stimulation [50], and all warming trials (tip, right, left) were performed before all cooling trials.

Data Treatment

In order to compare perceived intensities of stimuli across individuals, data were rescaled relative to a non-taste sensation [55]. The remembered intensity of "brightness of the sun when looking directly at it", herein referred to as brightness, was used to normalize the data [61]. Each subject's brightness rating was divided by the group average for this remembered sensation, creating an individualized normalization factor by which ratings for taste and non-taste oral sensations, including PROP and temperature from thermal stimulation, were divided. As discussed below, thermal taste ratings were not normalized. All rating data also underwent a $\log_{10}$ transformation, with ratings of zero converted to 0.2, to allow for direct comparison between our thermal taste data and that obtained by Green and coworkers [49,50,53].

Following normalization, data were examined for extreme outliers, defined as intensity ratings more than 3 times the interquartile range above the 3rd quartile [69]. Subjects were excluded if all of their taste, astringency, and metallic intensity ratings were extreme outliers. The data from 4 subjects, (1 Caucasian female, 1 non-Caucasian female, 2 Caucasian males), were excluded using these criteria. A summary of the demographic measures for the final subject cohort is provided in Table 1.

Table 1. Summary of demographic measures for subjects.

PROP taster status (PTS) group: pNT = non-taster, pMT = medium-taster, pST = super-taster; thermal taster status (TTS) group: TT = thermal taster, TnT = thermal non-taster, other = individuals not categorized; C = Caucasian, nC = non-Caucasian.

			PTS			TTS		
		All subjects (N=122)	pNT	pMT	pST	TT	TnT	other
Males	N	40	13	22	5	8	14	18
	Age	18-60	19-55	18-60	24-43	19-55	21-50	18-60
	Mean	31.2	27.4	33.4	30.6	30.5	30.8	31.8
	C/NC	32/8	9/4	18/4	5/0	7/1	11/3	14/4
	Smokers	7	4	2	1	0	3	4
	TT/TnT	NA	4/2	2/11	2/1	NA	NA	NA
	pNT/pMT/pST	NA	NA	NA	NA	4/2/2	2/11/1	7/9/2
Females	N	82	25	39	18	16	35	31
	Age	18-68	18-54	18-64	18-68	18-54	18-64	18-68
	Mean	31.0	31.0	29.3	34.6	30.2	31.8	30.7
	C/NC	70/12	23/2	31/8	16/2	13/4	32/3	12/4
	Smokers	5	1	4	0	0	2	2
	TT/TnT	NA	6/15	5/15	5/5	NA	NA	NA
	pNT/pMT/pST	NA	NA	NA	NA	6/5/5	15/15/5	4/19/8

PROP Taster Status (PTS) and Thermal Taster Status (TTS) Categorization

PTS categorization of normalized data was achieved using the cut-off values employed by Porubcan and Vickers [61], as the current study applied the same normalization factor. The duplicate PROP intensity ratings were averaged, and PTS groupings determined as pNTs <10.9 mm; pMTs, 10.9-61.5 mm; and pSTs >61.5 mm.

In order to maintain the position of "weak" on the gLMS, which was used to categorize individuals, thermal taste data were not normalized. TTs were defined as those that reported the same taste sensation, rated above weak, at the same location and temperature trial in both replicates [50]. In order to establish homogeneous TTS groupings, TnTs were defined as those that did not perceive any taste sensation in any trial.

Statistical Analysis

All analyses were performed with SPSS 16 (SPSS Inc., IL, USA). Correlations between all measures were examined using Pearson's r with a minimum significance level defined as $p<0.05$. PTS and TTS effects on perceived intensity of oral stimuli were examined separately using one-way, repeated measures, fixed-model ANOVA with replicates as the within-subjects variable and PTS or TTS as the between-subjects variable. A two-way, repeated measures, fixed-model ANOVA was used to examine the interaction between PTS and TTS on the perceived intensity of oral sensations. Main effects of gender, ethnicity, and smoking on the intensity of oral sensations, and the interactions of PTS and TTS with gender, ethnicity, and smoking were examined separately using 2-way, repeated measures, fixed-model ANOVA. In these analyses, PTS or TTS, gender, ethnicity, and smoking were between-subjects variables and replicates was the within-subjects variable. Tukey's HSD was used as the mean separation test following a significant ANOVA.

Results

Main Effects

6-n-propylthiouracil (PROP)

6-n-propylthiouracil (PROP) intensity was treated as a continuous variable allowing for examination of its correlation with FP density, age, and intensity ratings for all oral stimuli. All perceived temperature, taste, astringency, and metallic flavour intensity ratings were significantly and positively correlated with PROP intensity ratings (Table 2). PROP ratings were also significantly correlated with FP density, while age and salivary flow rate (SFR) were not associated with PROP intensity ratings.

PROP Taster Status (PTS)

PROP taster status (PTS) categorization yielded 38 PROP non-tasters (pNTs; 13 males, 6 non-Caucasian, 5 smokers), 61 medium-tasters (pMTs; 22 males, 12 non-Caucasian, 6 smokers), and 23 super-tasters (pSTs; 5 males, 2 non-Caucasian, 1 smoker; Table 1).

One-way repeated measures ANOVA was performed, and means and Tukey's HSD results are summarized in Figure 1. Low ($F(2, 119 = 9.92, p<0.001$) and high ($F(2, 119 = 13.50, p<0.001$) astringency, low ($F(2, 119) = 12.11, p<0.001$) and high ($F(2, 119) = 14.47, p<0.001$) metallic, bitter ($F(2, 119) = 9.04, p<0.001$), sweet ($F(2, 119) = 18.86, p<0.001$), salty ($F(2, 119) = 5.08, p<0.01$), and sour ($F(2, 119) = 8.37, p<0.001$) were rated significantly more intense by pSTs than either of the other PTS groups. Perceived warmth on the tip ($F(2, 119) = 14.17, p<0.001$), right ($F(2, 119) = 5.71, p<0.01$) and left ($F(2, 119) = 6.50, p<0.01$), and coolness on the tip ($F(2, 119) = 5.62, p<0.01$), right ($F(2, 119) = 7.14, p<0.01$) and left ($F(2, 119) = 4.43, p<0.05$) were also significantly more intense for pSTs than either pNTs or pMTs. No significant differences were found between pNTs and pMTs. One-way ANOVA revealed that SFR did not differ significantly between the 3 PTS groups ($F(2, 119) = 0.73, p = 0.49$; data not shown).

Table 2. Correlations between perceived PROP intensity and oral stimuli, fungiform papillae density (FP/cm^2), age, and salivary flow rate.

Temperature stimuli are indicated as temperature quality-tongue location, where W = warmth, C = coolness; bolded values are significant at the 0.01 level; (ns) = non-significant.

	Prototypical Tastants			
	Sweet	Sour	Salty	Bitter
PROP Bitterness	**0.554**	**0.327**	**0.293**	**0.350**
	Metallic		Astringent	
	Low	High	Low	High
PROP Bitterness	**0.465**	**0.458**	**0.469**	**0.411**
	Temperature					
	W-tip	W-right	W-left	C-tip	C-right	C-left
PROP Bitterness	**0.503**	**0.407**	**0.419**	**0.381**	**0.417**	**0.372**
	Physiological Measures		
	FP/cm^2	Age	Salivary Flow
PROP Bitterness	**0.386**	0.134 (ns)	-0.09 (ns)

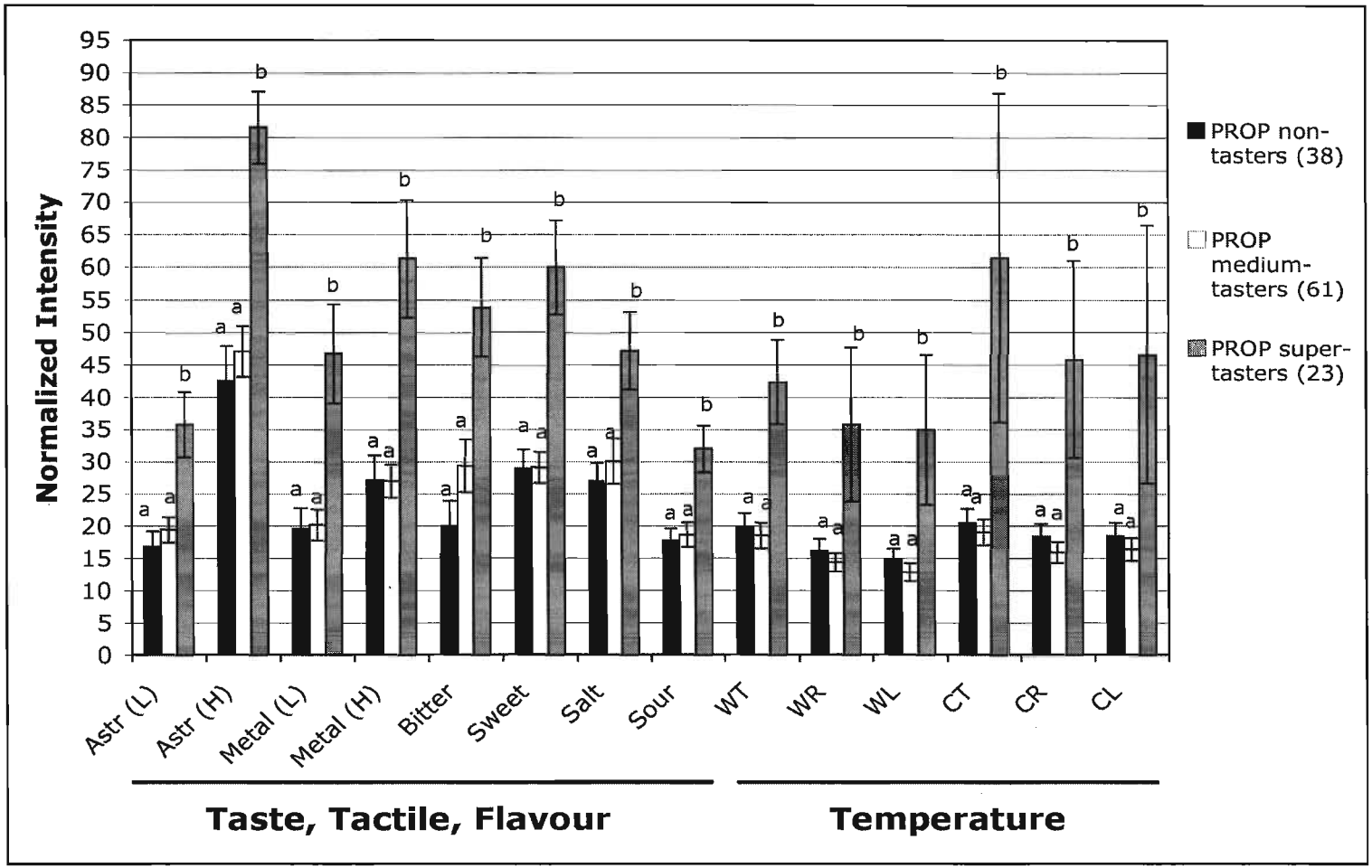


Figure 1. PROP taster status (PTS) effect on oral sensation.
Bars represent mean (normalized) intensity ratings +/- SE mean. Means with different letters differ at the $p<0.05$ level of significance. Astr, Astringency; Metal, Metallic; (L) = low level, (H) = high level; WT, warmth on the tip of tongue; WR, warmth on the right; WL, warmth on the left; CT, coolness on the tip; CR, coolness on the right.

Thermal Taste

Due to the stringent categorization criteria used to determine thermal taster status (TTS) groups, 49 subjects were excluded from the analysis. Of the remaining 73 subjects, 24 were categorized as thermal tasters (TTs; 8 males, 5 non-Caucasians, no smokers) and 49 were categorized as thermal non-tasters (TnTs; 14 males, 6 non-Caucasian, 5 smokers; Table 1).

One-way repeated measures ANOVA was performed on the normalized data, and means are presented in Figure 2. Although TTs rated all oral stimuli higher in intensity that TnTs, only ratings for low astringency ($F(1, 71) = 6.15$, $p<0.05$), high metallic ($F(1, 71) = 5.69$, $p<0.05$), and warmth on the left ($F(1, 71) = 8.98$, $p<0.01$) were significant. Warmth on the right fell just short of significance ($F(1, 71) = 3.69$, $p = 0.059$), and no difference in SFR was found between the two groups ($F(1, 71) = 0.45$, $p = 0.50$; data not shown).

One-way repeated measures ANOVA was repeated on logged data to allow direct comparison with Green and coworkers [49,50,53]. TTs rated almost all stimuli as higher in intensity than TnTs (Figure 3), including low ($F(1, 71) = 9.23$, $p<0.01$) and high ($F(1, 71) = 7.86$, $p<0.01$) astringency, high metallic ($F(1, 71) = 6.95$, $p<0.05$), bitter ($F(1, 71) = 3.86$ $p<0.05$), sweet ($F(1, 71) = 6.77$, $p<0.05$), salty ($F(1, 71) = 9.49$, $p<0.01$), warmth on the tip, ($F(1, 71) = 12.70$, $p<0.001$), right ($F(1, 71) = 5.03$, $p<0.05$) and left ($F(1, 71) = 13.49$, $p<0.001$), and coolness on the right ($F(1, 71) = 5.81$ $p<0.05$) and left ($F(1, 71) = 7.01$, $p<0.01$). Coolness on the tip approached significance ($F(1, 71) = 3.93$, $p = 0.051$). Ratings for sour ($F(1, 71) = 3.08$, $p>0.05$), low metallic ($F(1, 71) = 3.56$, $p>0.05$), and PROP did not differ ($F(1, 71) = 0.04$, $p>0.05$).

*PROP Taster Status*Thermal Taster Status*

Of the 24 TTs, 10 were pNTs, 7 were pMTs, and 7 were pSTs. Of the 49 TnTs, 17 were pNTs, 26 were pMTs, and 6 were pSTs (Table 1). In 2-way repeated measures ANOVA, no interactions were found between PTS and TTS for any oral stimuli ($p(F)>0.05$). As only extreme, homogeneous groups of TTS were used for analysis, an additional analysis was carried out that excluded pMTs, the most variable and

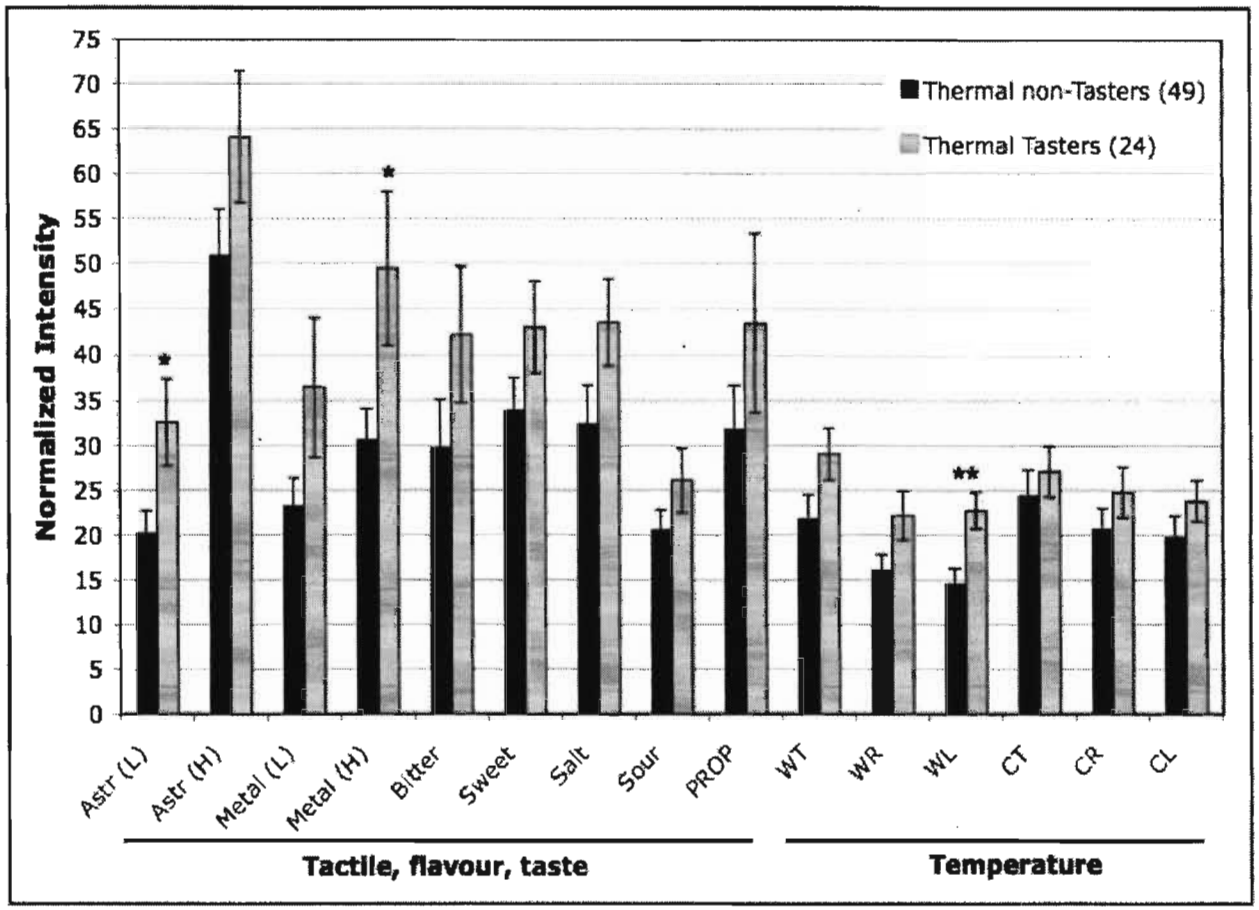


Figure 2. Thermal taster status (TTS) effect on oral sensations.
Bars represent mean (normalized) intensity ratings +/- SE mean. Astr, Astringency; Metal, Metallic; (L) = low level, (H) = high level; PROP, 6-n-propylthiouracil; WT, warmth on the tip of tongue; WR, warmth on the right; WL, warmth on the left; CT, coolness on the tip; CR, coolness on the right; **significantly different at $p<0.01$, *significantly different at $p<0.05$.

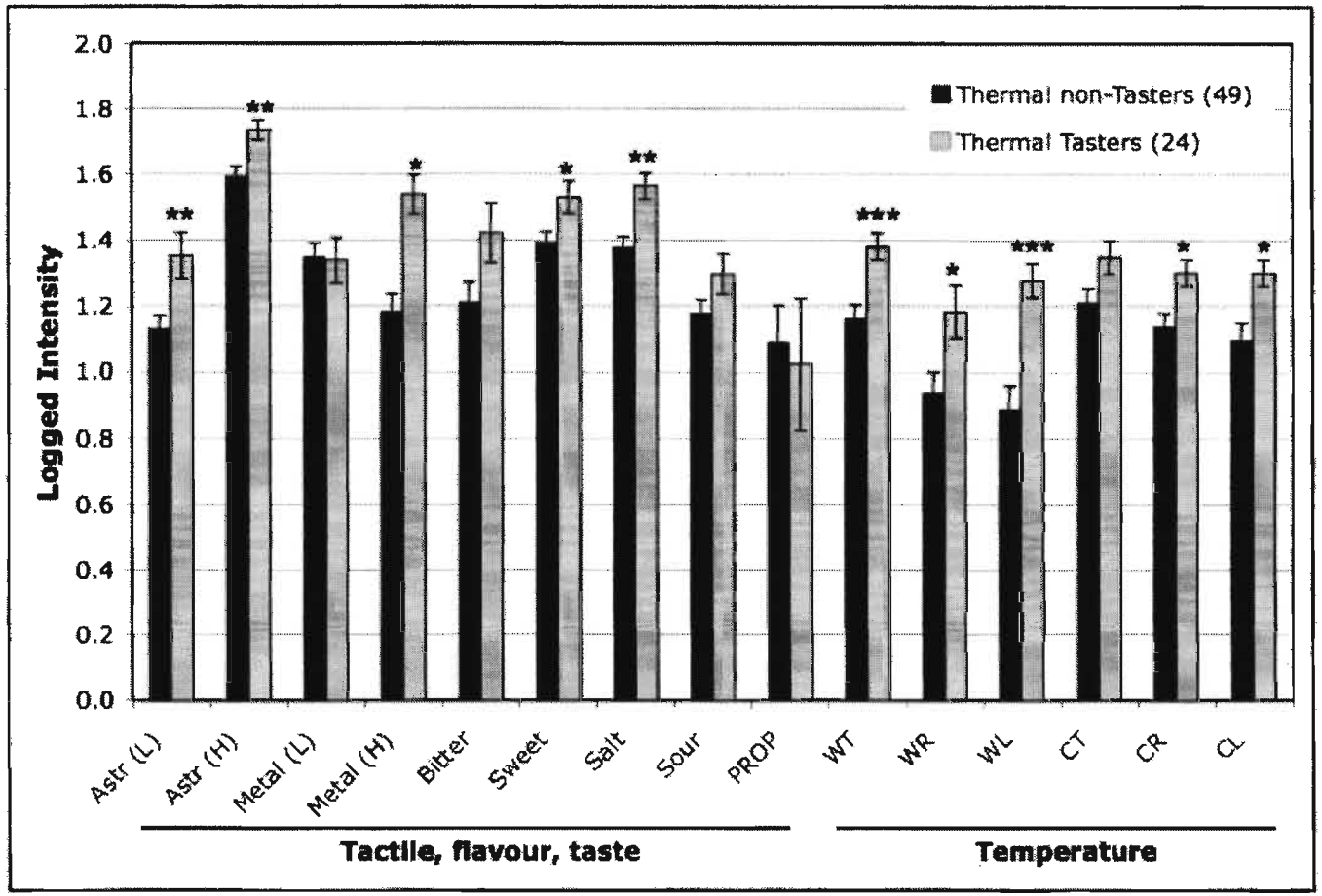


Figure 3. Thermal taster status (TTS) effects on logged intensity ratings of oral stimuli.
Bars represent mean (logged) intensity ratings +/- SE mean. Astr, Astringency; Metal, Metallic; (L) = low level, (H) = high level; PROP, 6-n-propylthiouracil; WT, warmth on the tip of tongue; WR, warmth on the right; WL, warmth on the left; CT, coolness on the tip; CR, coolness on the right. ***<0.001, ** p<0.01, * p<0.05.

difficult PTS group to categorise. Again, no interactions between PTS and TTS were found using two-way repeated measures ANOVA ($p(F)>0.05$).

Further Considerations

FP density, age, smoking, SFR, gender, and ethnicity were examined for potential effects on oral sensation intensity and interaction with the main factors under investigation. The same PTS and TTS main effects were significant in the 2-way repeated measures ANOVAs described below as in the 1-way repeated measures ANOVAs described above.

Fungiform Papillae (FP) Density

Fungiform papillae (FP) density was determined for 111 subjects, including 36 pNTs, 55 pMTs, 20 pSTs, 23 TTs, and 43 TnTs. FP density was weakly and positively correlated with PROP intensity (Table 2) and perceived warmth on the tip of the tongue ($r = 0.225$, $p<0.05$). One-way ANOVA revealed significant differences in FP density between PTS groups, with pSTs having the greatest density, followed by pMTs, and then pNTs ($F(2, 108) = 14.968$, $p<0.001$; Figure 4). No significant difference was found between female and male FP density ($F(1, 109) = 1.195$, $p>0.05$). FP density was not associated with TTS nor was it correlated with age ($r = -0.06$, $p>0.05$) or SFR ($r = -0.01$, $p>0.05$).

Age & Smoking

Age was not correlated with any of the oral sensations examined ($p(r)$ and $p(F)>0.05$), and did not differ as a function of PTS or TTS ($p(F)>0.05$). Smoking was not associated with the perceived intensity of oral sensations, and no interactions were found between smoking and PTS or TTS ($p(F)>0.05$).

Salivary Flow Rate (SFR)

The average SFR was 2.64 g/min+/-1.03SD, with a range of 1.07 g/min to 6.48 g/min. SFR was not correlated with any of the oral stimuli examined ($p(r)>0.05$). A scatter-plot of the data was created (not shown) to determine whether there was a natural divide that could be used to categorize subjects into high- and low-flow groups, however no such break in the data was found. Thus, the cohort was divided into 2 equal sized groups of

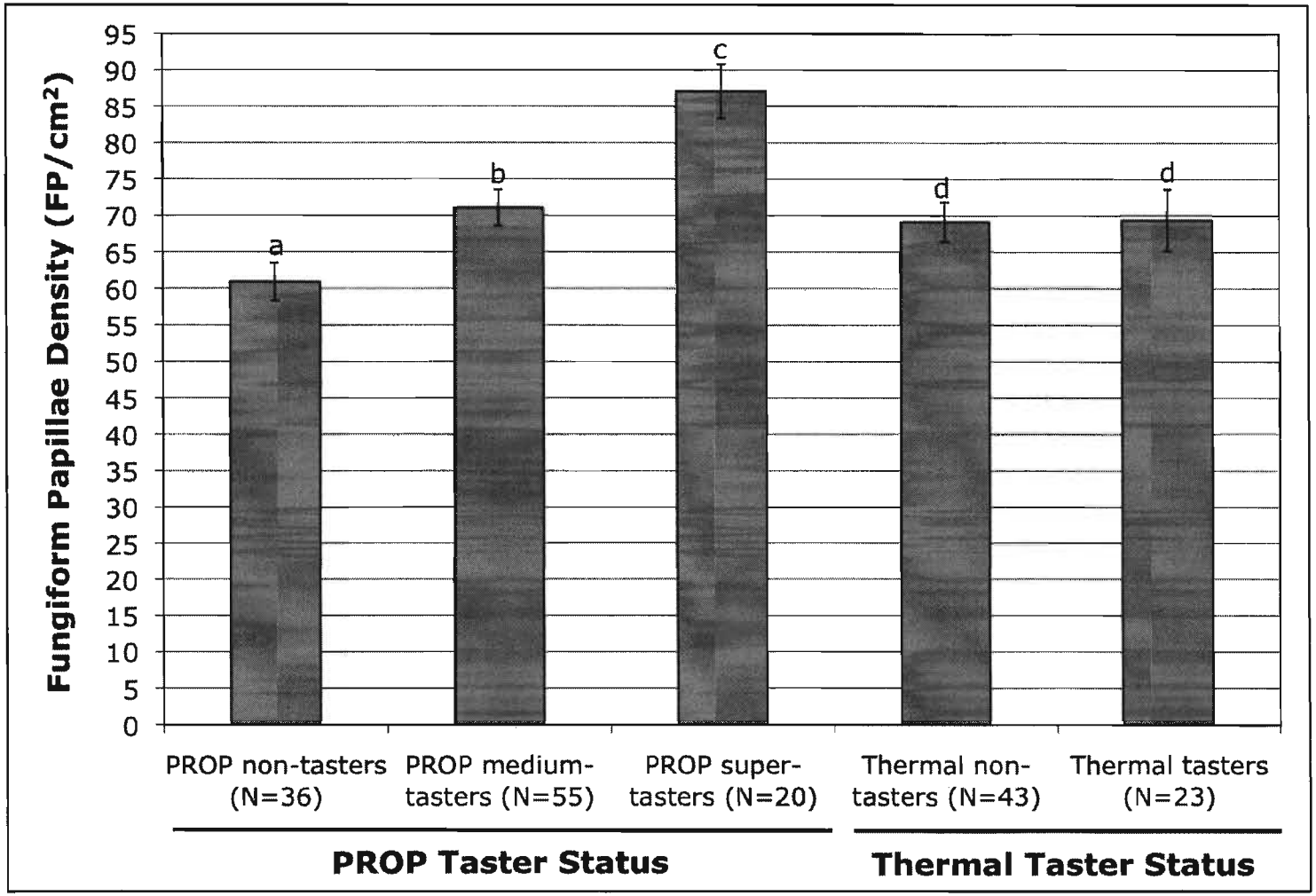


Figure 4. Fungiform papillae density (FP/cm^2) for PROP taster status (PTS) and thermal taster status (TTS) groups.
Bars represent mean (normalized) intensity ratings +/- SE mean. Means within each taster group with different letters differ at the $p<0.05$ level of significance.

61, creating a low-flow (M = 1.89+/-0.37) and a high-flow (M = 3.43+/-0.85SD) group. These two flow groups did not differ in their oral stimuli intensity ratings ($p(F)>0.05$). To further examine SFR, extreme SFR groups were established by dividing subjects into quartiles and excluding data from the 2nd and 3rd quartiles, creating 2 groups of 30 subjects (data not shown). One-way repeated measures ANOVA revealed no differences between these low- and high-flow groups in ratings for all oral sensations ($p(F)>0.05$).

Gender, Ethnicity, and PTS Interactions

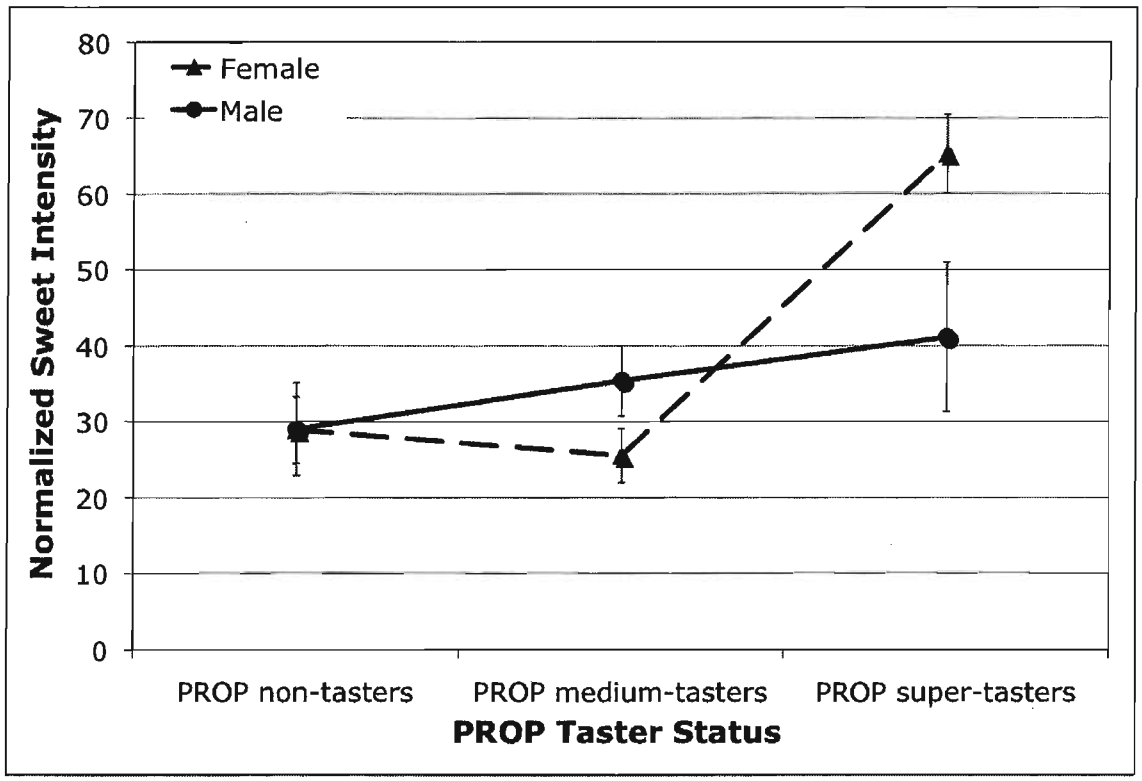
No significant main effects of gender were found ($p(F)>0.05$). A significant PTS*gender interaction was shown for sweet intensity ($F(2, 116) = 3.70$, $p<0.05$; Figure 5a). PTS*gender interactions for high astringency ($F(2, 116) = 2.92$, $p = 0.058$; Figure 5b) and warmth on the tip ($F(2, 116) = 3.06$, $p = 0.051$; Figure 5c) approached significance. As there were only 5 male pSTs, caution must be taken in interpreting this result, however, the trend of female pSTs rating oral sensations higher than male pSTs held for all sensations except sour and high astringency.

A significant main effect of ethnicity on perceived intensity of salt was found ($F(1, 116) = 4.11$, $p<0.05$), with non-Caucasians (M = 48.03+/-7.12SE) rating salt intensity higher than Caucasians (M = 32.67+/-2.59SE).

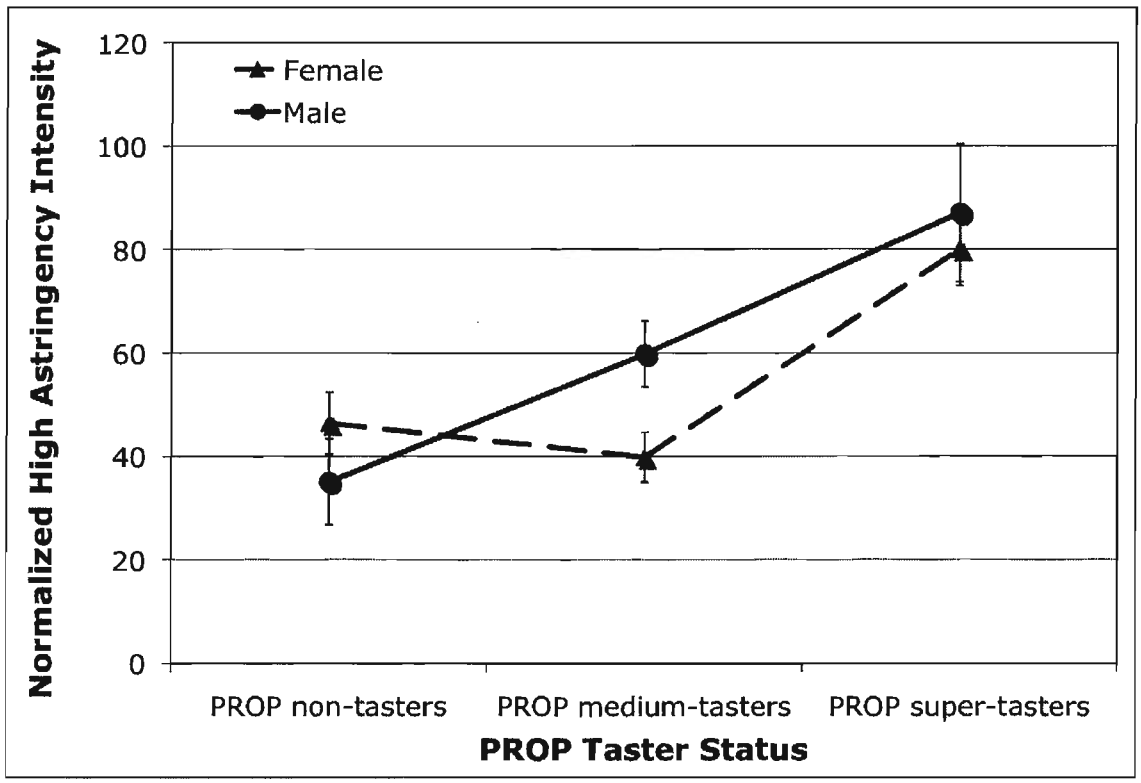
Gender, Ethnicity and TTS Interactions

No significant effects of gender were found ($p(F)>0.05$). A significant TTS*gender interaction was shown for salt ($F(1, 69) = 6.06$, $p<0.05$), with female TTs (M = 48.60+/-6.81SE) rating the intensity higher than their TnT (M = 26.40+/-4.60) counterparts, and male TTs (M = 33.48+/-9.62) rating it lower than male TnTs (M = 47.21+/-7.28).

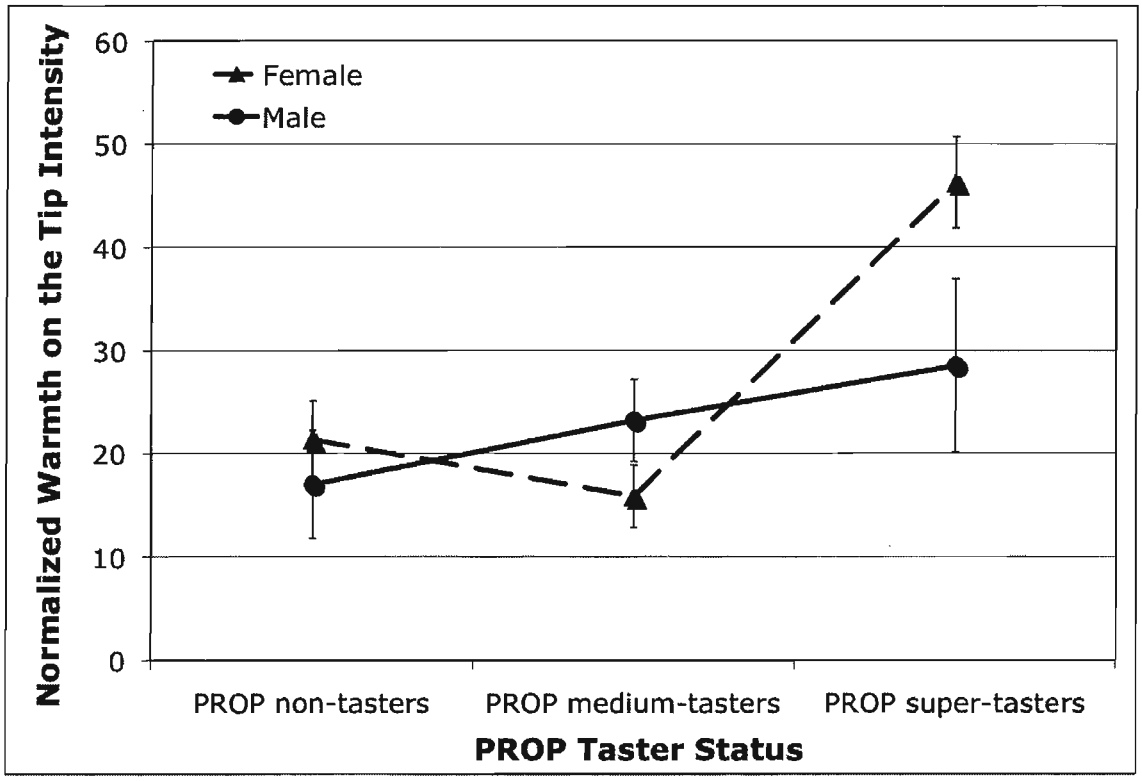
Ethnicity had a significant main effect on ratings for low astringency ($F(1, 69) = 6.30$, $p<0.05$). Caucasians rated low astringency higher in intensity (M = 29.47+/-2.63SE) than non-Caucasians (M = 13.55+/-5.77SE). TTS*ethnicity interactions for low astringency ($F(1, 69) = 3.86$, $p=0.054$; data not shown) and low metallic ($F(1, 69) = 3.91$, $p=0.052$; data not shown) approached significance. Although there were only 5 non-Caucasian TTs, a trend was observed of non-Caucasian TTs rating the intensity of all oral sensations lower than non-Caucasian TnTs. Interestingly, the opposite trend was


Female
Male
Normalized Sweet Intensity
80
70
60
50
40
30
20
10
0
PROP non-tasters
PROP medium-tasters
PROP super-tasters
PROP Taster Status

A)


Female
Male
Normalized High Astringency Intensity
120
100
80
60
40
20
0
PROP non-tasters
PROP medium-tasters
PROP super-tasters
PROP Taster Status

B)



C)

Figure 5. PROP taster status (PTS)*gender interaction for a) sweet intensity, b) high astringency intensity, and c) warmth on the tongue tip. Line symbols represent mean (logged) intensity ratings +/- SE mean.

observed with Caucasians, where TTs rated all oral sensations as more intense than TnTs.

Discussion

The PTS group proportions reported here (31% pNTs, 50% pMTs, 19% pSTs) approached that previously reported for North American populations (25% pNTs, 50% pMTs, and 25% pSTs) [18], although there was a disproportionate number of pNTs. This may have resulted from the categorization criteria, as the PROP concentration used here was 10x lower than that used by Porubcan and Vickers [61], from which the PTS group criteria were taken. However, PTS groups obtained via scatter-plots and employing the criteria of Tepper et al. [70] yielded similar results (data not shown). The proportion of subjects categorized as pNTs agrees with the reported 32% Caucasian female pNTs in [71], which might be expected since approximately 2/3 of our subjects were Caucasian females.

In agreement with previous studies, pSTs rated all prototypical tastants higher in perceived intensity than both pMTs and pNTs [17-19]. pMTs did not have an advantage over pNTs, as no differences in tastant intensity were observed between the two groups. The lack of a difference between these two groups may be due to the concentration of PROP used, or the use of a single PROP solution for categorization [72,73]. pSTs also rated the astringency of both low and high concentrations of alum with greater intensity than pMTs and pNTs, which is in agreement with previous work using alum [27], and tactile stimuli in general [24-26]. This finding suggests that previous contradictory data [62,74] may have been due to less sensitive PTS categorization methods [65], as none of these previous studies employed the gLMS or normalization techniques. Iron sulfate's metallic flavour is driven by retronasal aroma while having little to no perceivable orthonasal aroma or astringency, and, at the concentrations used here, it is indistinguishable from water with nasal occlusion [59,63]. Interestingly, pSTs rated the metallic flavour intensity of both the low and high concentrations of iron sulfate significantly higher than pMTs and pNTs, consistent with other retronasal stimuli [27]. Yackinous and Guinard [24] reported lower orthonasal thresholds for diacetyl and

phenylethyl methyl ethyl carbamide in pSTs. Overall, these results may suggest a pST advantage in perception of olfactory stimuli, regardless of the delivery route.

FP are surrounded, and sometimes inhabited by trigeminal innervation [44,45], which carries temperature-sensitive free nerve endings [46]. Since pSTs have greater numbers of FP, it follows that pSTs may perceive temperature with greater intensity than pMTs and pNTs. pSTs rated warmth and coolness higher than either of the other two PTS groups. To our knowledge, this finding is novel and contradicts previous work that reported either no difference in lingual temperature responsiveness between PTS groups [75] or only modest differences that were not confined to the tongue [76]. Differences in thermal stimulation techniques and PTS grouping methods may account for the disparity between our respective results. While Manrique and Zald [76] employed a similar stimulation methodology to that used here, the plate of their thermal probe was larger (1.5 cm^2 vs. 0.64 cm^2, respectively), and their rate of temperature change was faster (0.5°C/s vs. 1°C/s, respectively). Also, they employed the LMS, which can produce a 'ceiling effect' that inhibits the ability of pSTs to accurately report the perceived intensity of sensations [55].

TTS effects on logged prototypical tastant intensities were as expected based on the work of Green and coworkers [49,50,53], with TTs rating all taste sensations higher than TnTs. Our divergent sour and PROP intensity results could be due to the different acids (citric acid vs. tartaric acid) and PROP concentrations (0.056 mM vs. 0.32 mM) used by the two groups. While Green et al. [53] did not find a thermal taste effect for irritating chemesthetic stimuli, we found that TTs rated the astringency of both concentrations of alum higher than TnTs. This suggests that perceived intensities of chemesthetic and tactile stimuli cannot act as predictors for each other. Given the different mechanisms involved in the transduction of chemesthetic and tactile stimuli (see [77] and [65], respectively, for review), this would be expected. The TT advantage for olfaction previously reported [50] was corroborated here using iron sulfate delivered retronasally. The higher rating of temperature sensations by TTs agrees with [50].

In order to examine potential interactions between TTS and PTS, TTS effects on normalized intensity scores of oral sensations were also determined. Interestingly, the trends were very similar to those observed using logged intensities, with the exception

of low metallic and PROP. Differences between normalized and logged data may be explained by the greater variability in the former. It has been suggested that data collected with the gLMS should be logged for analysis, as it is typically log-normally distributed across subjects [53]. Given the importance of sensory data to a range of disciplines, including psychology, food science, and health, and the desirability of direct comparison of results from different studies, it would be useful for scale and data treatment techniques to be more consistent between investigators. In light of the variability in TTs' ratings of the oral stimuli observed here, a detailed examination of TT sub-groups and uncategorized subjects may be beneficial.

Unexpectedly, we did not observe a PTS*TTS interaction for any of the stimuli presented. Previous data has shown that TTs rate PROP intensity significantly higher than TnTs [50]. Additionally, for all sensations except warmth on the left side of the tongue, the Eta squared (η^2) values are larger for PTS than for TTS, suggesting that PTS has a greater effect on perceived intensity (Table 3). The absence of association between PTS and TTS here implies that these two indices of oral responsiveness function via independent mechanisms, and may be under separate genetic control. The current study suggests a global sensory advantage for both pSTs and TTs, as they rate sensations across multiple modalities (i.e., gustatory, tactile, and olfactory) with greater intensity than their non-taster counterparts. This presents the possibility that a central nervous system gain mechanism, with peripheral indicators such as thermal taste and PROP intensity, may account for the heightened perceptions of pSTs and TTs [53]. As indicated in Green et al.'s [53] model of neurophysiological processes, emotional reactivity, and cognitive and response processes play a role in perception by modulating central responses. Recent work demonstrating that pSTs' respond to negatively charged stimuli with significantly higher emotional reactivity than pNTs [78] suggests that the emotional and cognitive states and processes of pSTs, and perhaps TTs, deserve greater consideration.

Further Considerations

The expected relationship between PTS and FP density was observed [2,24,26,40,79]. Overall FP density was within previously reported ranges

Table 3. Eta squared values for PROP taster status effects (PTS η^2) and thermal taster status effects (TTS η^2) on normalized intensity ratings.

	PTS η^2	TTS η^2
Astringency – Low	0.142	0.080
Astringency – High	0.185	0.029
Metallic – Low	0.088	0.048
Metallic – High	0.196	0.074
Bitter	0.132	0.025
Sweet	0.231	0.029
Salt	0.079	0.034
Sour	0.123	0.025
Warmth-tip	0.192	0.037
Warmth-right	0.088	0.049
Warmth-left	0.098	0.112
Cool-tip	0.086	0.005
Cool-right	0.107	0.016
Cool-left	0.069	0.016

[12,13,25,26,79], and was not associated with age or gender [41]. Also as expected, FP density was not correlated with whole-mouth ratings for prototypical tastants [12,41].

The lack of an association between TTS and FP density is a novel finding that provides evidence for the assertion that TTS is not mediated by innervation density [50], or presumably receptor density [40]. Recent data from knockout mice strongly suggests that thermal taste is mediated in part by the TRPM5 non-selective cation channel [52], which is critical for sweet, umami, and bitter transduction [51,80]. TRPM5's activation by intracellular calcium [81] presents the possibility that TTS effects on taste perception may result directly from differences in membrane depolarization and cell communication. This possibility is particularly attractive in light of the finding that type II taste receptor cells in mice lack the conventional machinery necessary for neurotransmission, but they do have TRPM5 channels [82]. However, it remains to be determined whether TRPM5 channels play as critical a role in human taste as they do in the mouse, and whether variants of the TRPM5 channel confer differential taste sensitivities in humans.

SFR did not associate with the perceived intensity of any oral stimuli, which was unexpected, particularly for PROP and astringency ratings. Previous studies have reported that either low-flow [66,83,84], or high-flow [85] groups rate the astringency of polyphenols higher. However, our results are in accordance with others that have found no relationship between alum astringency and SFR [74,86]. Evidence suggests that alum and polyphenols elicit astringency through different mechanisms [87], which may account for the difference in SFR effects on their perceived intensities. While it has been reported that high SFR is associated with higher PROP intensity ratings [88], our data shows no such relationship, which may result from differences in the stimuli used to elicit salivary flow.

Age was not associated with intensity ratings for any oral sensation, however, only 4 subjects were over 60, which is the approximate age when decreases in gustatory perception are expected to occur [3,89]. Also, a whole-mouth rinse was used here, which may mitigate age effects on taste intensity [90]. No effect of gender was observed, in agreement with Finkentscher et al. [91] who reported no effects of gender

on taste perception in subjects under the age of 40, but in contrast with [89,92], particularly for PROP intensity [2,93]. Smoking did not influence perceived intensity of oral stimuli, as previously reported [94], although the young median age of our smokers (25 years), may account for this finding [89,95]. With most of our non-Caucasian cohort being Asian, the effect of ethnicity on the perceived intensity of salt was expected based on the work of Bertino et al. [4].

Conclusion

pSTs and TTs possess greater responsiveness across taste, trigeminal, and retronasally presented olfactory stimuli. The categorization of pSTs, pMTs, and pNTs was corroborated by FP density, a physiological measure that appears to account for many of the differences in perception between PTS groups. For TTs, the increased responsiveness is independent of PTS and FP density, suggesting the phenomena of thermal taste and PROP tasting are genetically and mechanistically independent. The discovery of thermal taste [49] and recent reports of ST-like responses to other oral stimuli [60] present the possibility that a number of indices of individual variation in oral sensation exist that may be independent of PTS [12].

The results of the current work may have interesting implications for food/beverage preference and health. Examination of potential differences between TTS groups in their consumption behaviours and food and beverage preferences is currently underway, and may provide insight into the real-world significance of TTS. Similarly, the novel finding that both PTS and TTS affect the perceived temperature of lingual thermal stimuli may impact on food/beverage temperature preference, and general food preferences and behaviour.

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CHAPTER 4: INFLUENCE OF STIMULUS TEMPERATURE ON OROSENSATION AND VARIATION WITH TASTE PHEONOTYPE

Introduction

Stimulus Temperature

The effect of temperature on taste is readily apparent when one initiates consumption of a food or beverage while it is warm and continues or concludes consumption after it has cooled, or vice versa, and has been of interest to researchers for many decades. While there is general agreement between studies regarding the effect of temperature on taste sensitivity (McBurney et al., 1973; Paulus & Reisch, 1980), the influence of temperature on suprathreshold intensities of prototypical tastants has not been extensively examined, and cannot be inferred from threshold measures (Mojet et al., 2005; Bartoshuk et al., 1996).

The influence of temperature on suprathreshold measures of taste is complex, and studies to date have returned conflicting results. Moskowitz (1973) demonstrated that temperature does not affect the psychophysical functions of bitterness (quinine sulfate) or sourness (citric acid), but does have a positive relationship with salty (NaCl) and sweet (glucose) psychophysical functions. Green & Frankmann (1987) reported that the perceived intensity of saltiness (NaCl) and sourness (citric acid) were not affected by cooling. Astringency, a tactile sensation resulting from the interaction of astringent compounds and salivary proteins and the subsequent activation of mechanoreceptors in the oral surface (reviewed in Bajec & Pickering, 2008), appears to decrease with decreasing temperature (Peleg & Noble, 1999). However, this data comes from a study of cranberry juice, a complex beverage, and the small but significant decreases described were coincident with decreases in viscosity, a parameter known to affect astringency perception (Smith et al., 1996). To-date, the effect of temperature on the perceived intensity of a simple astringent solution has not been described. The most studied taste-temperature relationship is that for sweetness, which appears both tastant and concentration dependent. The sweetness of sucrose, glucose, and fructose increases with increasing temperature (Green & Frankmann, 1987; Bartoshuk et al., 1982; Green

& Frankmann, 1988), an effect that for sucrose diminishes with increasing concentration and becomes negligible at approximately 0.5 M sucrose (Green & Frankmann, 1987; Bartoshuk et al., 1982; Calvino, 1986). Saccharin, an artificial sweetener, appears not to be affected by temperature, while aspartame decreases in intensity with increasing temperature, an effect that also appears to depend on concentration (Schiffman et al., 2000).

Methodological Issues

Methodological discrepancies may account for some of the differences in the studies discussed above. While some used magnitude estimation (Moskowitz, 1973; Green & Frankmann, 1987; Bartoshuk et al., 1982; Calvino, 1986), others used time-intensity (Larson-Powers & Pangborn, 1978) or line scales (Schiffman et al., 2000). Recent studies examining scale use strongly suggest that the impact of individual variation must be taken into account, and that use of an appropriate top anchor is required to account for differences in scale use between subjects (Bartoshuk et al., 2002). Comparing data across studies that have used varying psychophysical techniques and reported different outcomes makes drawing general, overarching conclusions regarding the impact of temperature on the perceived intensity of taste both difficult and tenuous. Additionally, these studies differ in their integration of temperature into the experimental design and their method, or lack of tongue temperature control. Room temperature rinses are most commonly used (Moskowitz, 1973; Calvino, 1986), but the wording of one study suggests that rinses were optional and no rinse temperature is provided (Schiffman et al., 2000). Bartoshuk et al. (1982) attempted to maintain oral temperature at body temperature by using mouth temperature rinses after noting that a room-temperature rinse would cool the tongue past body temperature. Pangborn et al. (1970) further highlighted the importance of oral temperature control by demonstrating gradual downward- and up-shifts in oral temperature with the repeated sipping of cold or warm solutions, respectively. Green & Frankmann (1987) concisely noted that "...in a typical taste-temperature experiment the temperature of the tongue either changes rapidly and unpredictably when warm and cold stimuli are intermixed, or drifts slowly higher or lower when warm and cold stimuli are presented in blocks". This statement

underscores the need for appropriate experimental design paradigms and rinse protocols in these types of studies.

The influence of tongue temperature on the perceived intensity of taste was demonstrated in two studies examining the perceived intensity of sweet (sucrose, fructose, glucose) and bitter (caffeine) stimuli. Both sweet and bitter intensities were reduced when a cool solution was presented to a cooled tongue (Green & Frankmann, 1987), suggesting the effect of temperature was driven by tongue temperature rather than solution temperature (Green & Frankmann, 1987; Green & Frankmann, 1988). For bitterness, the greatest reduction was seen when the tongue was cooled to 20°C and the solution was 36°C, suggesting interesting taste and temperature interactions during the process of food ingestion where oral temperatures are in flux due to the different temperatures of foods and beverages being consumed sequentially (Green & Frankmann, 1987).

Thermal Taste

Recently, a novel temperature-taste interaction has been described. In approximately 20-50% of the population, heating or cooling small areas of the tongue elicits a taste sensation (Green & George, 2004; Bajec & Pickering, 2008). Typically, thermal tasters (TTs), who perceive phantom tastes evoked by thermal stimuli, report sweetness and saltiness upon warming and cooling, respectively, the tip of the tongue (Green & Cruz, 2000). On the lateral edges of the tongue, cooling yields sourness and/or saltiness (Green & Cruz, 2000). Bitterness and more complex sensations, such as metallic, have also been reported by TTs on either warming or cooling the tongue (Cruz & Green, 2000; Bajec & Pickering, unpublished observations). Thermal taste is also an indicator of individual variation in oral sensation, with TTs perceiving prototypical tastants (quinine sulfate, NaCl, citric acid, monosodium glutamate, sucrose), astringency (aluminum sulfate (alum)), and retronasal aromas (vanillin, iron sulfate) with greater intensity than thermal non-tasters (TnTs; (Green & George, 2004; Bajec & Pickering, 2008; Green et al., 2005). Evidence from *Trpm5* (transient receptor potential melastatin 5) channel knockout mice indicates that TRPM5, a TRP superfamily cation channel, may play a role in sweet thermal taste (Talavera et al., 2005), suggesting that

this source of individual variation is under genetic control. TRPM5 is involved in the transduction of umami, sweet, and bitter tastes, and has most recently been implicated in the complex percept resulting from iron sulfate ingestion in mice (Zhang et al., 2003; Damak et al., 2006; Riera et al., 2009). TRPM5 is temperature-sensitive and heat-activated, with inward currents increasing significantly between 15°C and 35°C (and declining between 35°C and 40°C) due to a temperature-dependent shift of the channel's activation curve (Talavera et al., 2005; Talavera et al., 2007). It is interestingly to note that, although it has yet to be determined whether basal levels of intracellular Ca^{2+} and heat alone can lead to TRPM5 activation (Talavera et al., 2005), the heating regimen used to elicit sweet thermal taste in humans is a ramp from 15°C to 40°C at approximately 1°C/s (Cruz & Green, 2000; Bajec & Pickering, 2008). Further, the augmentation of the chorda tympani response to sweet stimuli when delivered in conjunction with elevated temperatures (35°C) is abolished in *Trpm5* knockout mice (Talavera et al., 2005). This finding presents the possibility that TRPM5 is responsible for the reported influence of heating on the perceived sweetness of solutions discussed above. Indeed, TRPM5 is postulated to act as a coincidence detector of warm temperatures and stimuli that increase intracellular Ca^{2+}, such as tastant binding (Talavera et al., 2005).

Interestingly, Moskowitz (1973) reported that the perceived intensities of sweet (glucose), salty (NaCl), bitter (quinine sulfate), and sour (citric acid) peaked at 35°C and dropped off at lower and higher temperatures. While all processes involved in taste transduction are temperature dependent, the temperature dependence of salty, bitter, sour, and sweet responsiveness is thought to result from the temperature dependence of the channels responsible for their transduction (reviewed in Talavera et al., 2007). The activation of these channels by temperature may also account for the reporting of salty, sour, and bitter as thermal tastes (Cruz & Green, 2000; Talavera et al., 2007).

PROP Responsiveness

While the genetics of PROP tasting has not been conclusively elucidated (Hayes et al., 2008), PROP responsiveness is a well-researched, genetically-mediated index of individual variation in oral sensation (Reed et al., 1999; Drayna et al., 2003). PROP

intensity data is collected as a continuous variable, but PROP responsiveness is typically expressed categorically as PROP taster status (PTS), which consists of three groups: PROP super-tasters (pSTs), PROP medium-tasters (pMTs), and PROP non-tasters (pNTs) (Bartoshuk, 1993), which is particularly useful for examining data using analysis of variance (ANOVA; Bajec & Pickering, 2008). Besides PROP, PTS is also associated with responsiveness to other orosensory stimuli. pSTs perceive prototypical tastants (e.g., salt, sugar, acid), including other bitterants, with greater intensity than pNTs (Bajec & Pickering, 2008; Hayes et al., 2008; Tepper et al., 2009; Bartoshuk et al., 1998; Prescott et al., 2001). Irritation from ethanol (Duffy et al., 2004; Bartoshuk et al., 1993; Prescott et al., 2000; Duffy, 2004; Bartoshuk et al., 1994) and the tactile sensation of astringency (Bajec & Pickering, 2008; Pickering et al., 2006) are also perceived with greater intensity by those that perceive PROP as more bitter. Recent evidence suggests that pSTs also perceive retronasal aroma, and thermal stimuli on the tongue surface more intensely than pNTs and pMTs (Bajec & Pickering, 2008). Interestingly, and surprisingly, there is minimal literature regarding the effects of PTS on the perception of orosensory stimuli over time, or the influence of PTS on orosensory stimuli at different temperatures. Previous studies suggest that TTS and PTS function independently, a finding that is re-examined in the current work.

Objectives & Hypotheses

The main objective of the current work was to examine the influence of TTS and PTS on the relationship between temperature and taste perception using time-intensity (TI) methodology, which provides insight into the dynamics of taste perception by tracking its intensity from onset through to extinction (Cliff & Noble, 1990). Further, TI methodology provides a novel view of the influence of these markers of individual variation in oral sensation, and allows determination of whether the greater perceived intensity of orosensory stimuli reported by TTs and pSTs extends to other parameters of oral sensation. The influence of TT subtype (i.e., TTs that perceive sweetness on warming the tongue, bitter on cooling or warming the tongue, and sourness on cooling the tongue) on TI parameters for the chemical tastant that elicits the corresponding taste was examined. We hypothesized that if TTs' taste transduction pathways are more

sensitive to temperature than TnTs, as suggested by their response to thermal stimuli, and they are more responsive to gustatory and astringent stimuli, they may present differences in TI parameters of taste when tastants are delivered in conjunction with thermal stimuli. Further, if the channels involved in the transduction of taste are taste/temperature coincidence detectors and are more sensitive to temperature in TTs, then the TI response of TTs to the tastant that chemically elicits the taste they perceive upon thermal stimulation (i.e., sweetness on warming, bitter on cooling, etc.) may differ at different temperatures. A secondary objective of this study was to contribute to the current understanding of the interaction between temperature and taste using TI methodology. Our goal here was to examine whether some of the discrepancies reported in the literature can be addressed by examining orosensory perception as a dynamic event, rather than a static snapshot.

Materials and Methods

Subjects

74 subjects were recruited from the student, staff, and faculty populations of Brock University, and from the local community. Subjects attended three sessions, the first lasting approximately 1.5 hours, and the remaining two lasting approximately 2.5 hours each. Subjects were instructed not to eat or drink anything for one hour prior to either session. When applicable, incentive was provided in the form of a credit toward a 1st year university Psychology course. During the first session, 30 individuals were excluded based on age (>45; 4), chronic use of prescription medication (2), smoker status (2) and not meeting TTS categorization criteria (22). 44 individuals completed both sessions. Incentive to complete the 3 sessions was provided in the form of a $20 gift certificate. Ethnicity was determined using the Census Canada "Ethnic Origin User Guide" (Statistics Canada, 2001). Thirty-nine individuals reported White as their ethnicity, 2 reported Chinese, 2 Black, and 1 South Asian. All procedures were approved by the Brock University Ethics Board (#08-006) and written consent was obtained from each participant.

Session 1: Scale acclimation, training, thermal and PROP taster status determination

Scale acclimation and training

Subjects used the generalized visual analogue scale (gVAS) to rate the perceived intensity of orosensory stimuli. Like the generalized labeled magnitude scale (gLMS; Bartoshuk et al., 2002; Bartoshuk et al., 2004), the gVAS offers the benefit of a top anchor that is outside of the modality of interest (i.e., strongest sensation ever experienced) while removing the potential limitations imposed by the 'strongest sensation imaginable' top anchor of the gLMS and its intermediate labels (Snyder et al., 2008; L. Bartoshuk personal communication, 2008). The gVAS is based on the VAS scale with "no sensation" as an anchor on the low extreme (0 mm) and "strongest sensation ever experienced" at the top extreme (100 mm). Three unlabelled and equidistant line anchors break the scale into four quarters (at 25, 50 and 75 mm, respectively). During the first session, subjects were asked to rate the intensity of each of 15 remembered sensations on the gVAS in order to familiarize them with the scale and ensure its proper usage (Bajec & Pickering, 2008). Each participant received both written and verbal instruction regarding proper scale usage. Aqueous solutions of taste and non-taste oral sensations were presented as aqueous solutions in order to familiarize participants with the sensations. Equi-intense stimulus concentrations were based on a review of the pertinent literature followed by bench testing. Subjects evaluated the intensity of labeled 20 ml samples representing the sweet (250 mM sucrose; Sigma-Aldrich, MO, USA), sour (3.25 mM citric acid; Fisher Scientific, ON, Canada), bitter (0.0275 mM quinine hydrochloride (quinine); Sigma-Aldrich, MO, USA), astringent (0.877 mM; aluminum sulfate (alum); Sigma-Aldrich, MO, USA) and umami (125 mM L-glutamic acid monosodium salt hydrate (MSG); Sigma-Aldrich, MO, USA) were presented in clear ISO glasses in random order. All solutions were made with pure water (Millipore RiOs 16 Reverse Osmosis System, MA, USA), stored in the dark at 3–4°C, and brought to room temperature (21± 2°C) well in advance of testing. Solutions were not kept for more than 7 days and were typically discarded within 5 days of preparation, with the exception of MSG, which was made fresh every two days to avoid off-flavour development. Subjects were instructed to take the entire sample, swirl for 5

s, expectorate, wait 10 s then rate the maximum intensity perceived on a gVAS scale. A minimum break of one minute was enforced between each sample. During this break participants were required to rinse with filtered water, a 5 g/L pectin solution (Pomona's Universal Pectin, MA, USA), then finally with filtered water again. Filtered water was also available to subjects *ad libitum*. After this exercise, and a short break during which subjects completed a demographic questionnaire, the same five stimuli were presented as 20ml samples in clear ISO glasses coded with three digit random numbers, and subjects were asked to identify the oral sensation elicited from a list of five possibilities (sweet, sour, bitter, astringent and umami) and rate its intensity. If a subject made an incorrect identification, the procedure was repeated a maximum of two times. All subjects correctly identified the oral sensations.

Subjects were then asked to rate the intensity of each of 15 remembered sensations on the gLMS in order to aid with familiarization and ensure its proper usage (Bajec & Pickering, 2008). Subjects received both written and verbal instruction on how to use the scale appropriately.

Thermal taster status (TTS) determination

Thermal taster status (TTS) was determined after Bajec & Pickering (2008). A 64 mm^2 computer-controlled Peltier device with a thermocouple feedback attached to a toothbrush-sized water-circulated heat sink (thermode) was applied to the subject's extended tongue by the researcher. Three locations on the edge of the tongue were stimulated discretely and in order: the most anterior tip, and approximately 1 cm to the right and then the left of the midline. Warming trials started at 35°C, cooled to 15°C, and re-warmed to 40°C (held for 1 s). The start temperature for cooling trials was 35°C, followed by cooling to 5°C (held for 10 s). Warming trials preceded cooling trials at each location to avoid possible adaptation from the intense, sustained cold stimulation (Green & George, 2004), and all warming trials (tip, right, left) were performed before all cooling trials (Bajec & Pickering, 2008). After each stimulation, participants were instructed to rate the intensity of any oral sensations perceived, including temperature, on 6 individual gLMSs labeled "temperature", "sweet", "salty", "sour", "bitter" and "other". The heating and cooling cycle was repeated on each of the three tongue

locations in a re-randomized order after a short break during which subjects completed demographic and health questionnaires. TTs were defined as those that reported the same taste sensation, rated above weak, at the same location and temperature in both replicates. Those that did not perceive any taste sensations in any trial were defined as TnTs. Subjects who did not meet either of these criteria were deemed uncategorizable and excluded from the study.

6-n-propylthiouracil (PROP) taster status (PTS) determination

At the end of the session, subjects rinsed with 10ml of a 3.2mM solution of PROP (MP Biomedicals; OH, USA) for 5 s, expectorated, and waited for 10 s before rating the maximum intensity of bitterness perceived on a gLMS scale (Porubcan & Vickers, 2005). This procedure was repeated at the end of the second session to obtain duplicate data. PROP taster status (PTS) was determined using the average rating of the two replicates, and groupings were: PROP non-tasters (pNTs) $\leq$22 mm; PROP medium tasters (pMTs), 22.1-51.9 mm; and PROP super-tasters (pSTs) $\geq$52 mm [40]. The frequency distribution of PROP ratings confirmed the appropriateness of these categorization values (data not shown).

Sessions 2, & 3: Time-intensity (TI) training and TI measures of prototypical tastants

In Session 2, use of the gVAS was reviewed, and subjects were trained in the time-intensity (TI) tasting protocol and use of the Compusense five 4.6™ (Compusense Inc., Guelph, ON) TI module using the gVAS. The sampling rate for TI data collection was 5 samples/s. This training consisted of subjects evaluating sweet, sour, bitter, and astringent samples at room temperature (20±1°C) using the TI software and taking the prescribed breaks and rinses. The instructions to subjects were to place the sample at their lips, click start on the TI software using the mouse and immediately take entire volume in mouth. As soon as the taste/sensation of interest was perceived, subjects initiated intensity rating. They were then to rinse gently with the sample until the instructions on the screen indicated it was time to expectorate (10 s). Ten seconds was chosen as the rinse time based on bench testing, which demonstrated that a rinse of this duration allowed adequate time in mouth after accounting for the time required for

stimulus delivery. Subjects continued rating the intensity post-expectoration, keeping the mouth closed and motionless, while allowing the tongue to move within the confines of the teeth. Subjects were instructed to rate only the specific taste being asked for. Subjects were instructed that the intensity rating should be zero when they no longer detected the specific taste/sensation being rated. Between each sample, a 2-min break was enforced, during which time subjects rinsed once with pectin once and at least twice with filtered water. Subjects were instructed to take a longer break if they felt it necessary. Filtered water was available *ad libitum* during breaks. Subjects were allowed to practice rating using the TI data collection system until they felt comfortable and confident with the task required.

At the start of Session 3, subjects were reminded of the use of the gVAS and the TI software. The remainder of Session 3 consisted of the subjects using TI to rate samples of sweet, sour, bitter, and astringent stimuli at the concentrations noted above at 5±0.2°C or 35±0.1°C. Samples were stored and presented as 20 ml aliquots in 2 oz glass jars with lids (Wheaton, Fisher Scientific, ON, Canada). Samples stored at 5°C were discarded if not used within 5 days of preparation, while samples stored at 35°C were discarded daily if not used to avoid microbial growth (Moskowitz, 1973). The desired serving temperature of the samples was achieved by cooling the sample in its presentation jar in a fridge set at or warming in a water-bath set for a minimum of 8 hours prior to use. Samples were presented in random order; while temperatures were blocked and balanced between subjects. Rinses of filtered water and pectin were provided at 35°C to maintain the buccal cavity near body temperature between samples (Bartoshuk et al., 1982). In an effort to simulate normal eating behaviour, the temperature of the tongue was not cold or warm adapted prior to testing. During the first half of the session, subjects rated all of the samples at one temperature, following the protocol outlined above. After a 15-min break, subjects rated the samples at the other temperature. At the end of the session, subjects repeated the PROP intensity rating.

Session 4 was followed the same protocol as session 3, however, the sample presentation temperature was reversed to that in session 3.

Statistical Analysis

The parameters generated by the Compusense TI module and their acronyms are listed in Table 1. Averaged data were examined for extreme outliers, defined as intensity ratings more than 3 times the interquartile range above the 3rd quartile (Kamerud & Delwiche, 2007; Bajec & Pickering, 2008). All statistical analysis was conducted using SPSS 15.0 software for Windows (SPSS Inc., Chicago, IL, USA). Paired t-tests were employed to examine the effects of temperature on TI parameters. Independent t-tests were used to examine the influence of TTS on each TI parameter for each taste/sensation at each temperature. One-way ANOVA was conducted to investigate the influence of PTS on each TI parameter for each taste/sensation at each temperature. The Boneferroni corrected p-value is 0.0063 when alpha is 0.05 and n=8 (number of TI parameters investigated per oral stimuli). All ANOVA and t-test results with a pre-Bonferroni p-level of 0.05 are reported. Two-way General Linear Model (GLM) fixed model ANOVA was used to examine TTS*PTS interactions and calculate Eta squared values (η^2) for PTS and TTS. Correlations between TI variables and the intensity of orosensory stimuli including PROP were examined using Spearman's ρ.

Results

A summary of demographics, and PTS and TTS distributions of this cohort is provided in Table 2.

TTS

Averaged curves for TTs and TnTs for each stimulus at each temperature are provided in Figure 1.

Temperature Effects Between TTS Groups

Independent t-tests were employed to examine the influence of TTS on TI parameters for each stimulus individually. No significant differences were observed between TTs and TnTs for any TI parameter (data not shown). A trend of TTs having greater

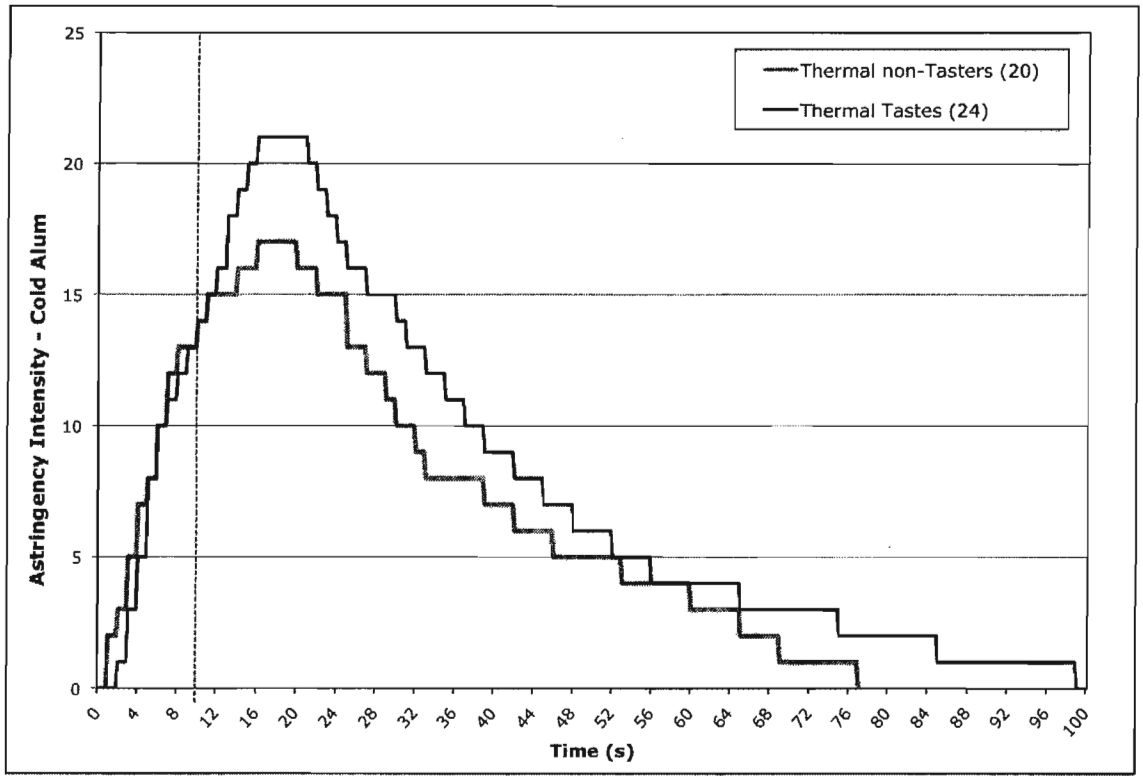
Table 1. Time-intensity parameters.

TMax	Time (s) to maximum intensity
IMax	Maximum intensity
DUR	Total duration (s) that the sensation is rated
AUC	Total area under the time-intensity curve
IAng	Angle (°) of sensation increase from start to IMax
IArea	Increase Area - area under the ascending portion of the curve from start to Imax
DAng	Angle (°) of sensation from IMax to the last recorded value
DArea	Decrease Area – area under the descending portion of the curve from Imax to the last recorded value
IDelay	Initial Delay – time (s) to first response
IInt	Initial Intensity – first intensity response

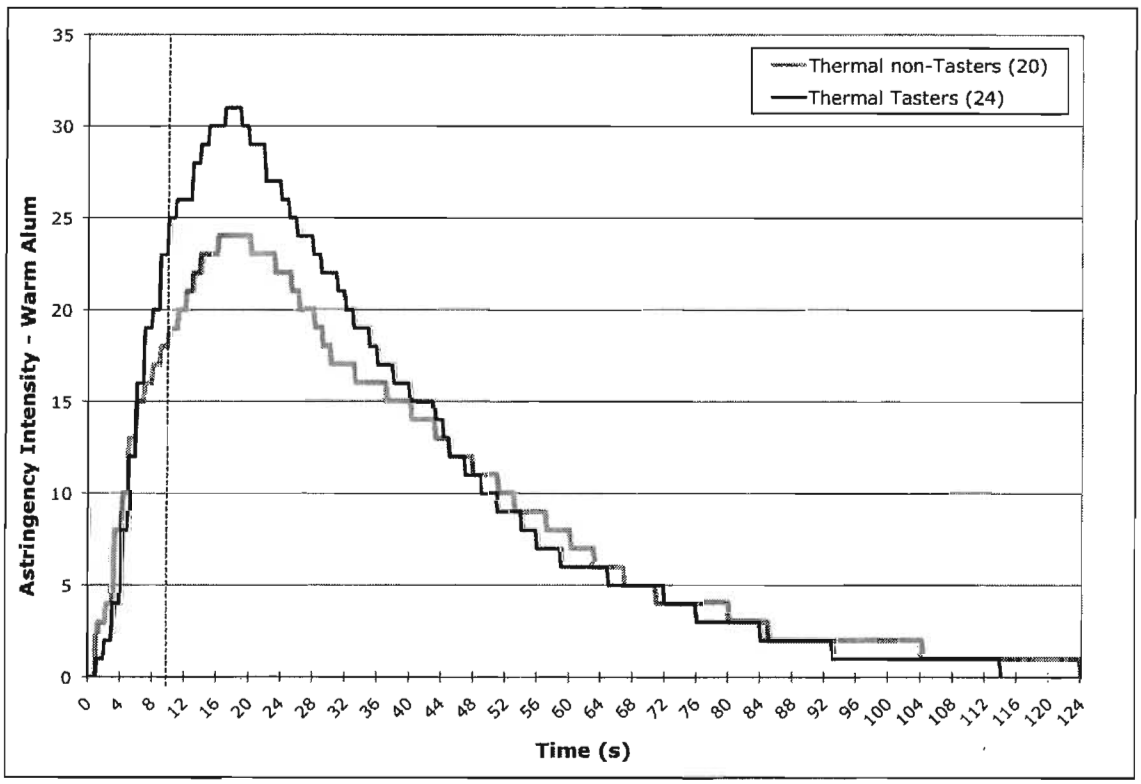
Table 2. Summary of demographic measures.

	PROP Taster Status			
Thermal Taster Status	**pNT**	**pMT**	**pST**	**Total**
TnT male/female (mean age: age range)	2/2 (28: 20-35)	5/7 (28: 22-44)	1/3 (22: 18-26)	8/12
TT male/female (mean age: age range)	3/5 (29: 24-40)	2/7 (27: 23-37)	2/5 (26: 19-35)	7/17
Total male/female	5/7	7/14	3/8	15/29

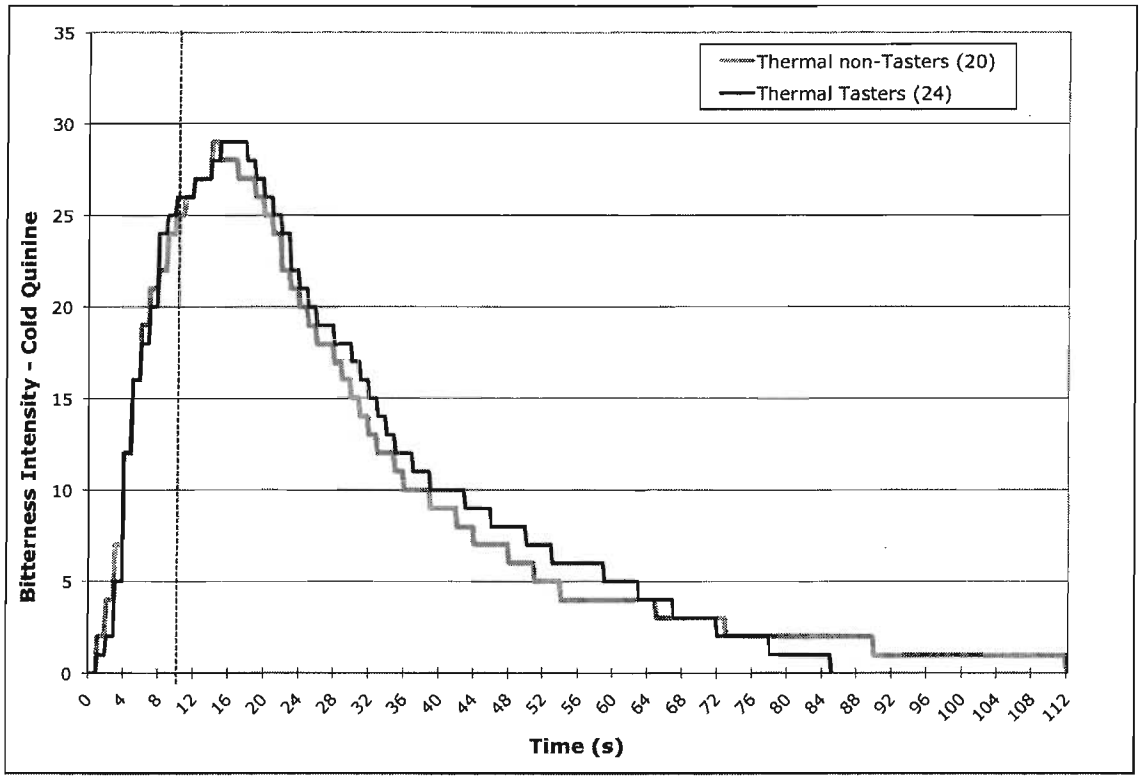
PROP = 6-n-propylthiouracil, pNT = PROP non-taster, pMT = PROP medium-taster, pST = PROP super-taster, TT = thermal taster, TnT = thermal non-taster


Thermal non-Tasters (20)
Thermal Tastes (24)
Astringency Intensity - Cold Alum
Time (s)

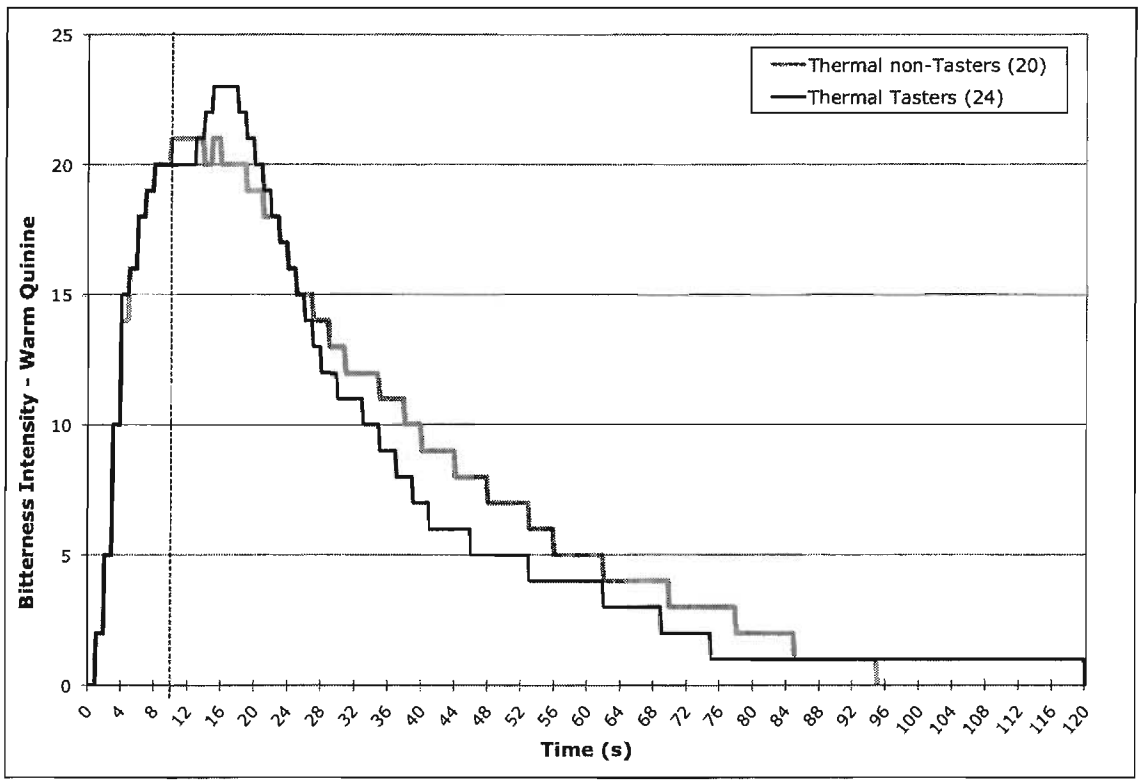
A)


Thermal non-Tasters (20)
Thermal Tasters (24)
Astringency Intensity - Warm Alum
Time (s)

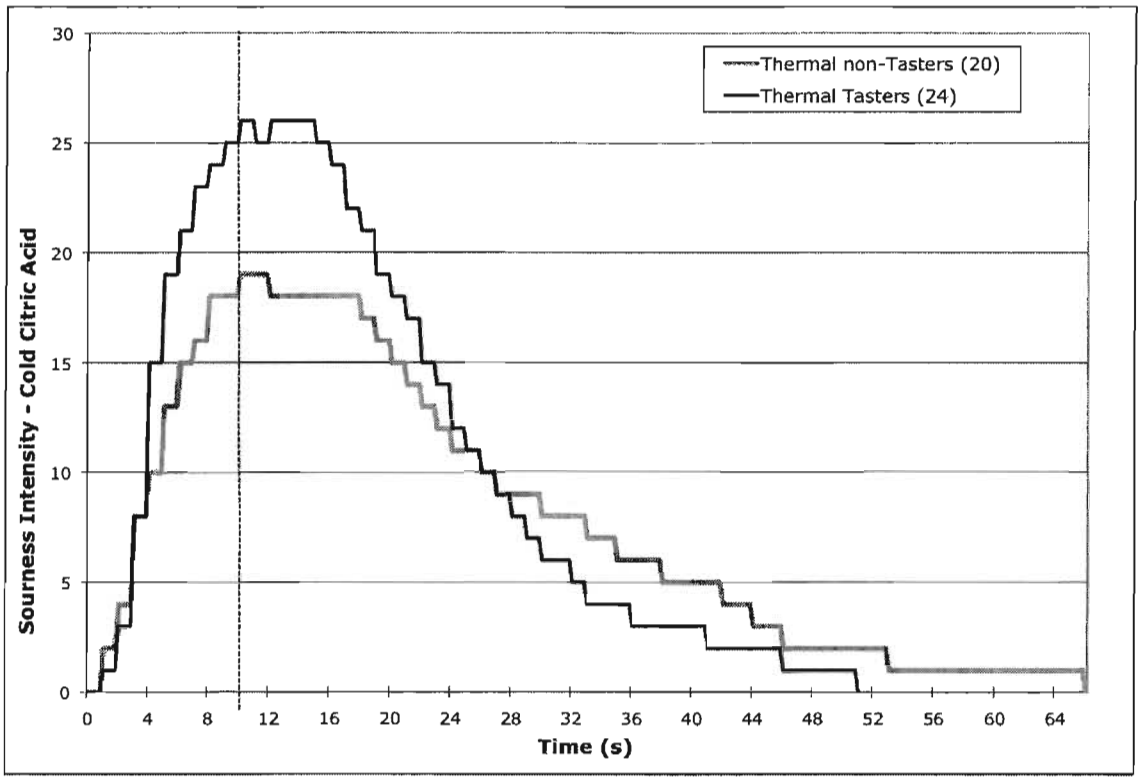
B)


Thermal non-Tasters (20)
Thermal Tasters (24)
Bitterness Intensity - Cold Quinine
Time (s)

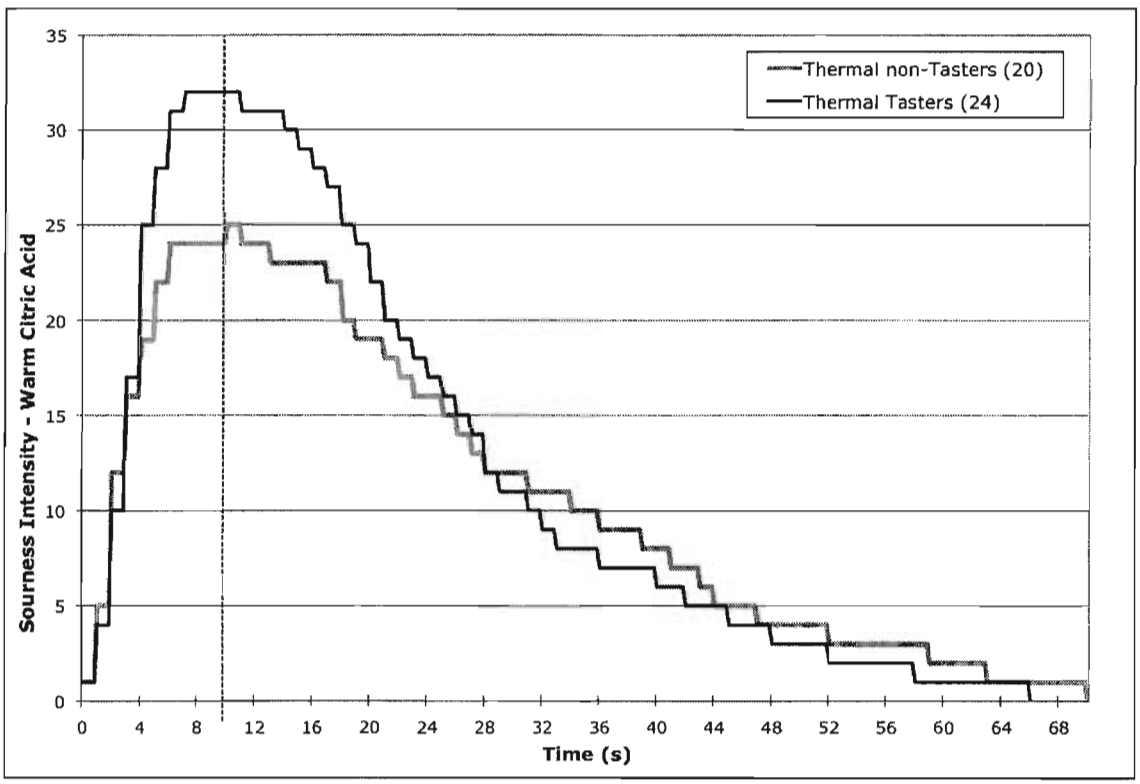
C)


Thermal non-Tasters (20)
Thermal Tasters (24)
Bitterness Intensity - Warm Quinine
Time (s)

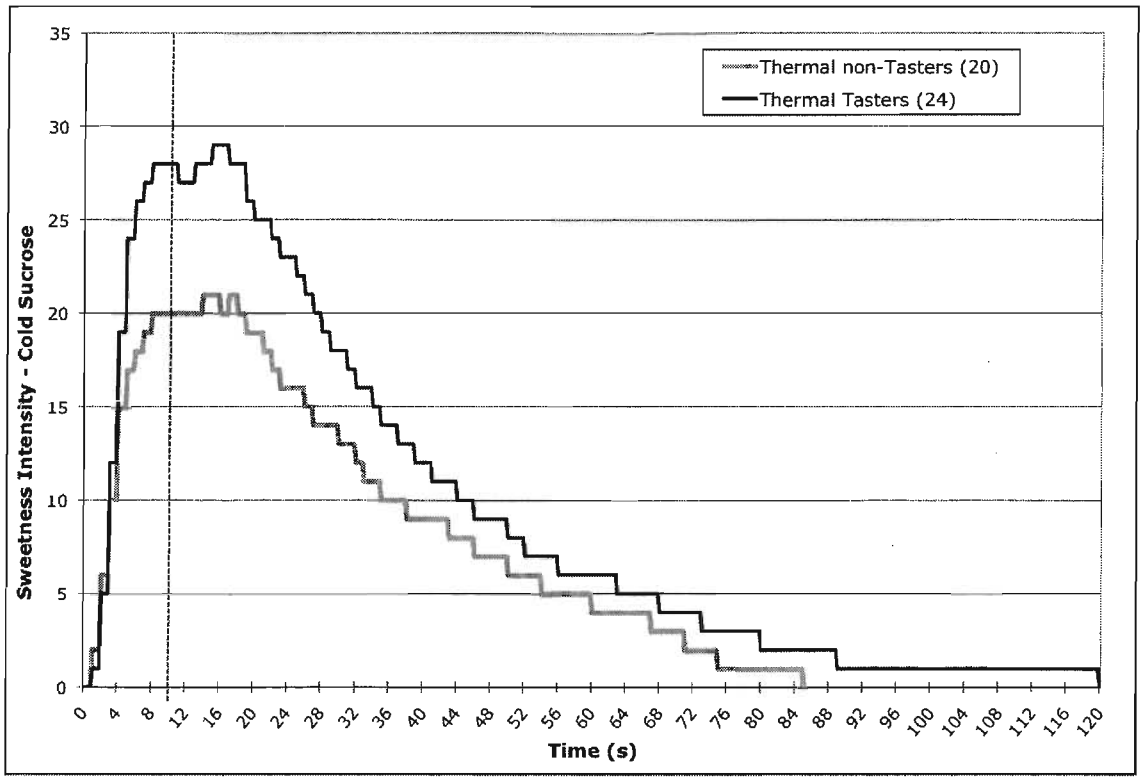
D)


Thermal non-Tasters (20)
Thermal Tasters (24)
Sourness Intensity - Cold Citric Acid
Time (s)
0
5
10
15
20
25
30
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64

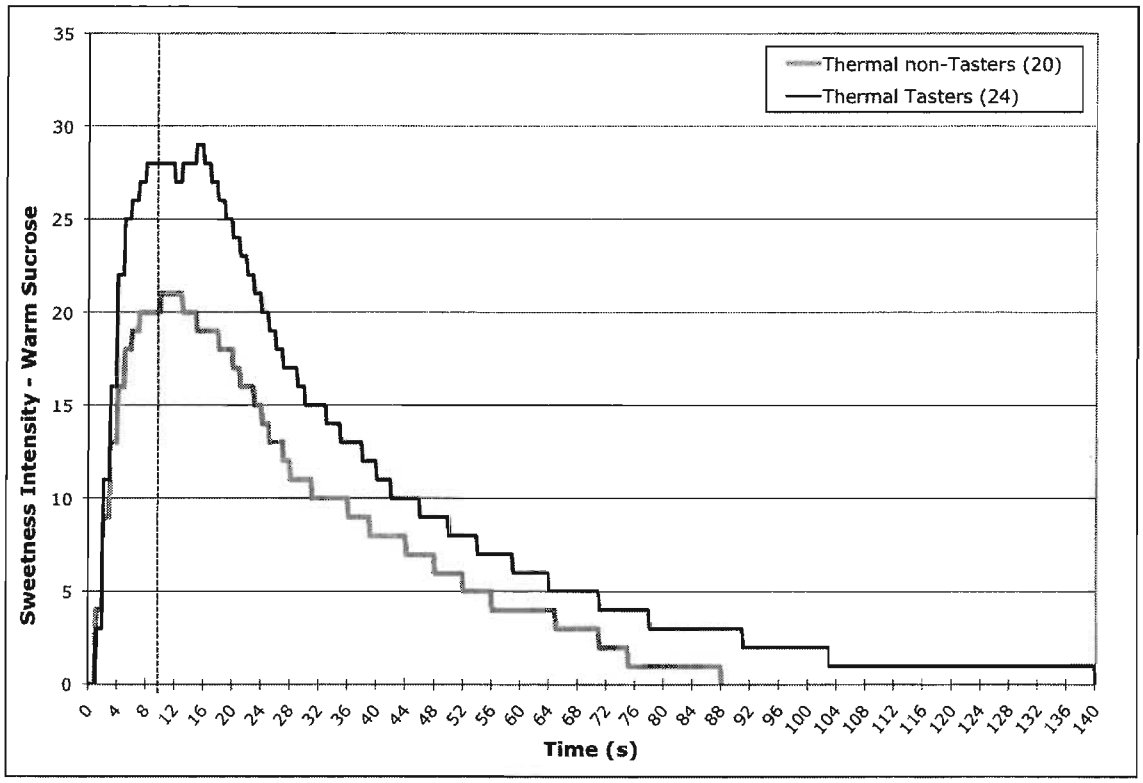
E)


Thermal non-Tasters (20)
Thermal Tasters (24)
Sourness Intensity - Warm Citric Acid
Time (s)
0
5
10
15
20
25
30
35
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68

F)


Thermal non-Tasters (20)
Thermal Tasters (24)
Sweetness Intensity - Cold Sucrose
Time (s)

G)


Thermal non-Tasters (20)
Thermal Tasters (24)
Sweetness Intensity - Warm Sucrose
Time (s)

H)

Figure 1. Thermal-taster and thermal non-taster averaged time-intensity curves for the perceived intensity of astringency from A) cold alum and B) warm alum, bitterness from C) cold quinine and D) warm quinine, sourness from E) cold citric acid and F) warm citric acid, and sweetness from G) cold sucrose, and H) warm sucrose. The vertical dashed-line at 10 seconds indicates sample expectoration.

maximum intensity (IMax), area under the curve (AUC), and decrease angle (i.e., area under the descending portion of the curve from Imax to the last recorded value; DAng), and longer time to maximum intensity (TMax) than TnTs for all stimuli at both temperatures was observed. Similarly, a trend of TTs having a greater increase angle (i.e., angle of sensation increase from start to IMax; IAng) for all warm stimuli, and a greater initial delay (i.e., time to first response; IDelay) for astringency and bitterness at both temperatures was observed. A trend of IDelay values for sour and sweet at both temperatures to be greater in TnTs than TTs was also observed.

Temperature Effects Within TTS Groups

Paired t-tests were employed to examine the influence of temperature on TI parameters in TnTs and TTs separately.

TnT

Increase area (i.e., area under the ascending portion of the curve from start to Imax; IArea) (t(19)=3.23, P=4.42E-3), DArea (t(19)=3.133, P=5.48E-3), Duration (i.e., total duration that the sensation is rated (Dur) (t(19)=4.05, P=6.90E-4), and IMax (t(19)=3.08, P=6.22E-4) were significantly greater for warm alum than cold. DAng (t(18)=3.21, P=4.83E-3) was greater for warm quinine than cold. IMax (t(19)=2.30, P=3.28E-2), Dur (t(19)=3.27, P=3.99E-3), AUC (t(19)=2.99, P=7.60E-3), and DArea (t(19)=2.92, P=8.88E-3) were greater for warm citric acid than cold. IAng (t(19)=2.86, P=1.00E-2), and IArea (t(19)=2.14, P=4.53E-2) were both greater for warm sucrose than cold.

TT

Warm alum IMax (t(23)=3.53, P=1.78E-3), Dur (t(22)=2.72, P=1.24E-2), AUC (t(22)=2.35, P=2.83E-2), and IArea (t(23)=2.68, P=1.35E-2) were greater than cold. TMax (t(23)=2.39, P=2.04E-2), and IArea (t(23)=2.43, P=2.34E-2) were both greater for cold quinine than warm. IMax (t(23)=2.47, P=2.13E-2), Dur (t(23)=2.86, P=8.89E-3), AUC (t(23)=2.91, P=7.99E-3), DArea (t(23)=2.77, P=1.10E-2), were greater for warm citric acid than cold. Cold citric acid TMax (t(23)=2.36, P=2.71E-2), IAng (t(23)=2.54, P=1.84E-2), and DAng (t(23)=2.49, P=2.06E-2) were greater than warm. TMax (t(23)=2.43, P=2.32E-2) and IArea (t(22)=1.06E-2) were greater for cold sucrose than warm.

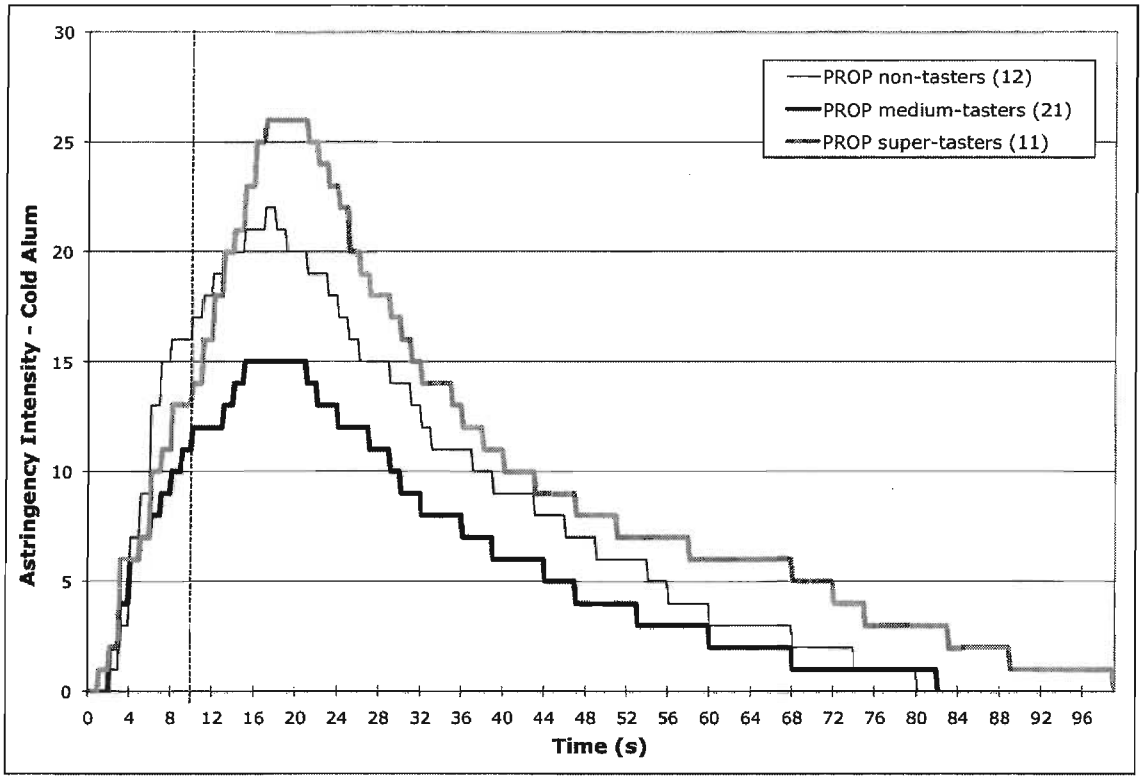
Additionally, a preliminary investigation of TT sub-groups was performed. Sub-groups of TTs were created based on the tastes they perceived during thermal stimulation. Sub-groups examined included TTs that perceived bitterness upon cooling (cold/bitter TTs; n=7), bitterness upon warming (warm/bitter TTs; n=5), sweetness upon warming (warm/sweet TTs; n=6), and sourness upon cooling (cold/sour TTs; n=9). Paired samples t-tests were used to examine whether TT sub-groups perceived the chemical tastant corresponding to the thermal taste they perceived differently depending on its temperature. Here we examined if, for example, cold/bitter TTs perceived cold quinine differently than warm quinine. The differences in TI parameters between warm and cold stimuli for the TT sub-groups were consistent with the differences observed in the cohort overall, and none were significant within TT sub-groups (data not shown). These results must be interpreted with caution, as there were a low number of TTs in each group and there was overlap of individuals between the groups.

PROP Bitterness and PTS

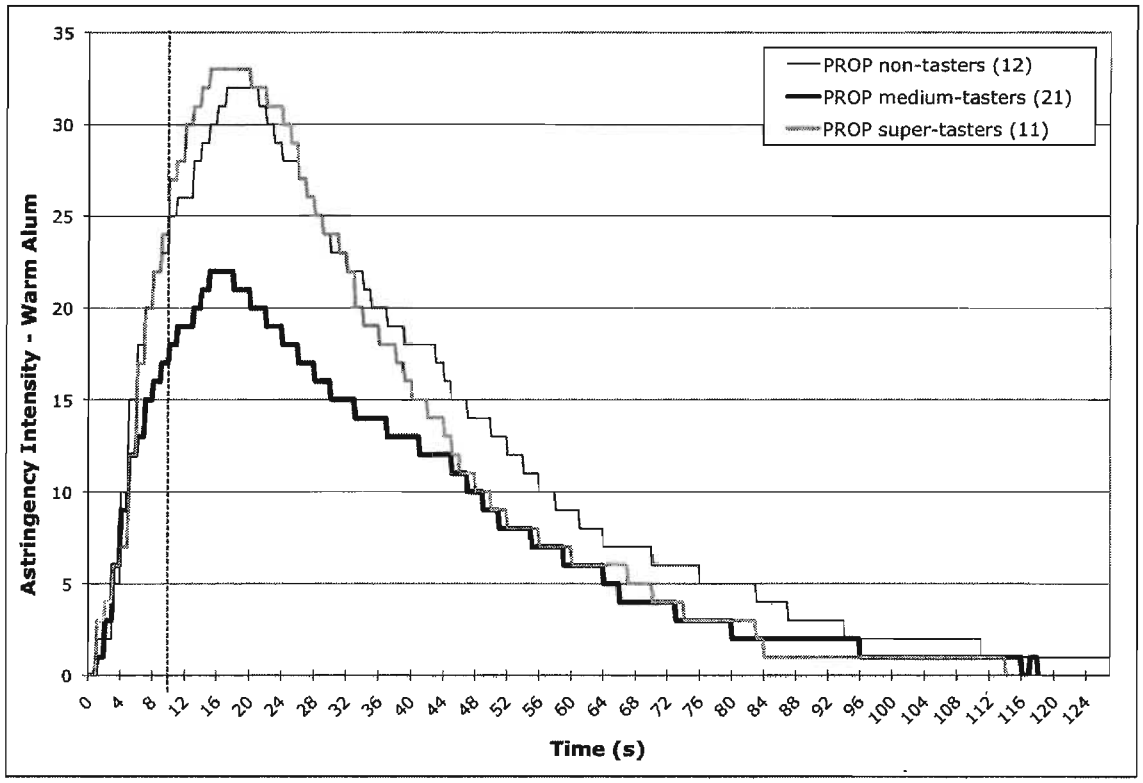
PROP bitterness intensity was significantly correlated with the Dur of cold sucrose ($r = -0.39$, $p<0.01$). PROP bitterness intensity was not significantly correlated with any other TI parameter (Appendix A). Averaged TI curves for pNTs, pMTs, and pSTs for each stimulus at each temperature are provided in Figure 2.

Temperature Effects Between PTS Groups

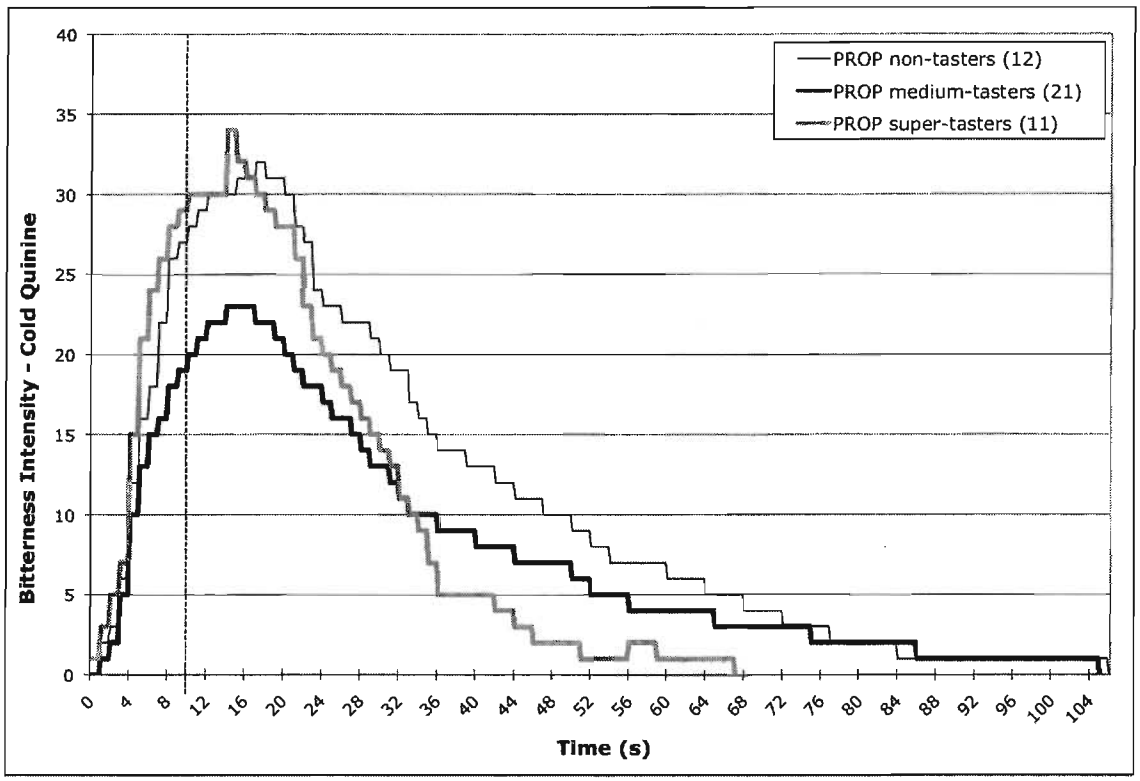
One-way ANOVA was employed to examine the influence of PTS on TI parameters for each stimulus individually. Initial intensity (IInt) for cold quinine ($F(2,43)=3.24$, $P=0.049$), and warm citric acid ($F(2,42)=4.12$, $P=0.024$) was significantly greater for pSTs than pMTs. IAng for cold citric acid ($F(2,43)=4.82$, $P=0.013$), cold sucrose ($F(2,43)=6.56$, $P=0.003$), and warm citric acid ($F(2,43)=4.87$, $P=0.013$) was significantly greater for pSTs than pMTs. IAng for warm quinine ($F(2,43)=5.53$, $P=0.007$) was significantly greater in pMT than pSTs, while for cold quinine ($F(1,43)=3.52$, $P=0.039$) it was significantly greater for pSTs and pNTs than pMTs. Warm quinine TMax was significantly longer for pNTs than pSTs ($F(2,43)=5.28$, $P=0.009$). pSTs rated the IMax of cold alum ($F(2,43)=4.32$, $P=0.02$), cold quinine ($F(2,43)=4.37$, $P=0.019$), warm alum ($F(2,43)=3.54$, $P=0.038$), warm citric acid


PROP non-tasters (12)
PROP medium-tasters (21)
PROP super-tasters (11)
Astringency Intensity - Cold Alum
Time (s)
0
5
10
15
20
25
30
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96

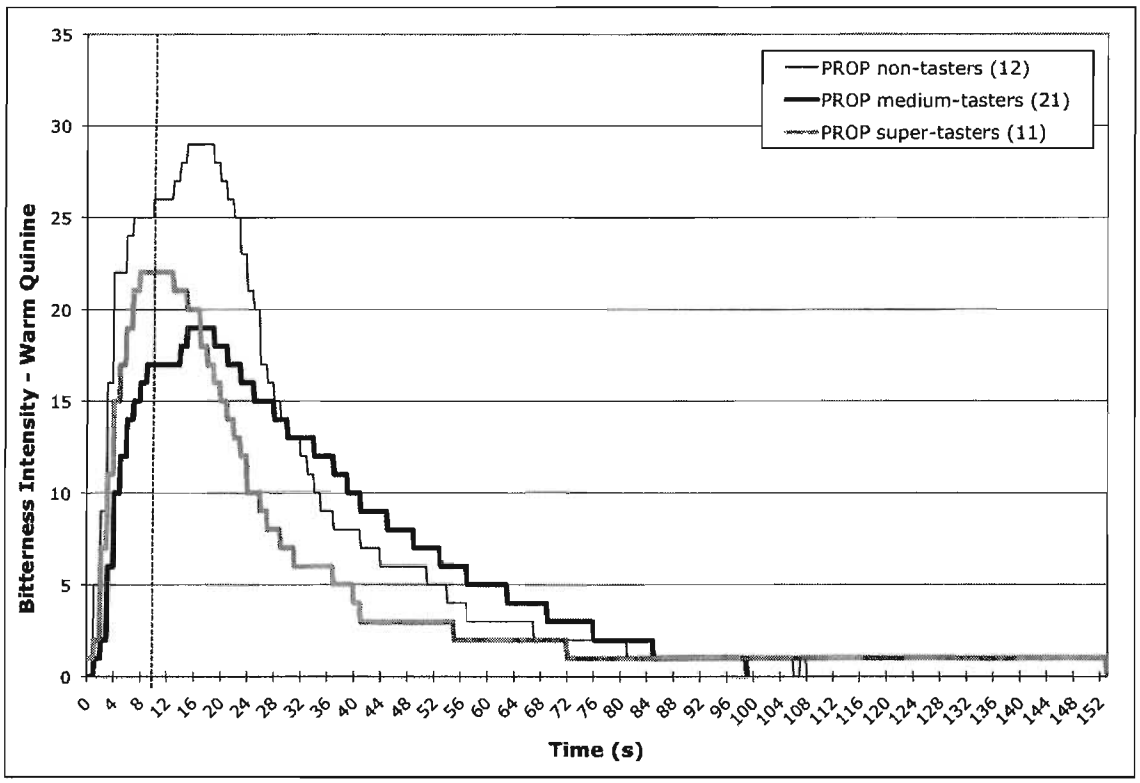
A)


PROP non-tasters (12)
PROP medium-tasters (21)
PROP super-tasters (11)
Astringency Intensity - Warm Alum
Time (s)
0
5
10
15
20
25
30
35
0 4 8 12 16 20 24 28 32 36 40 44 48 52 56 60 64 68 72 76 80 84 88 92 96 100 104 108 112 116 120 124

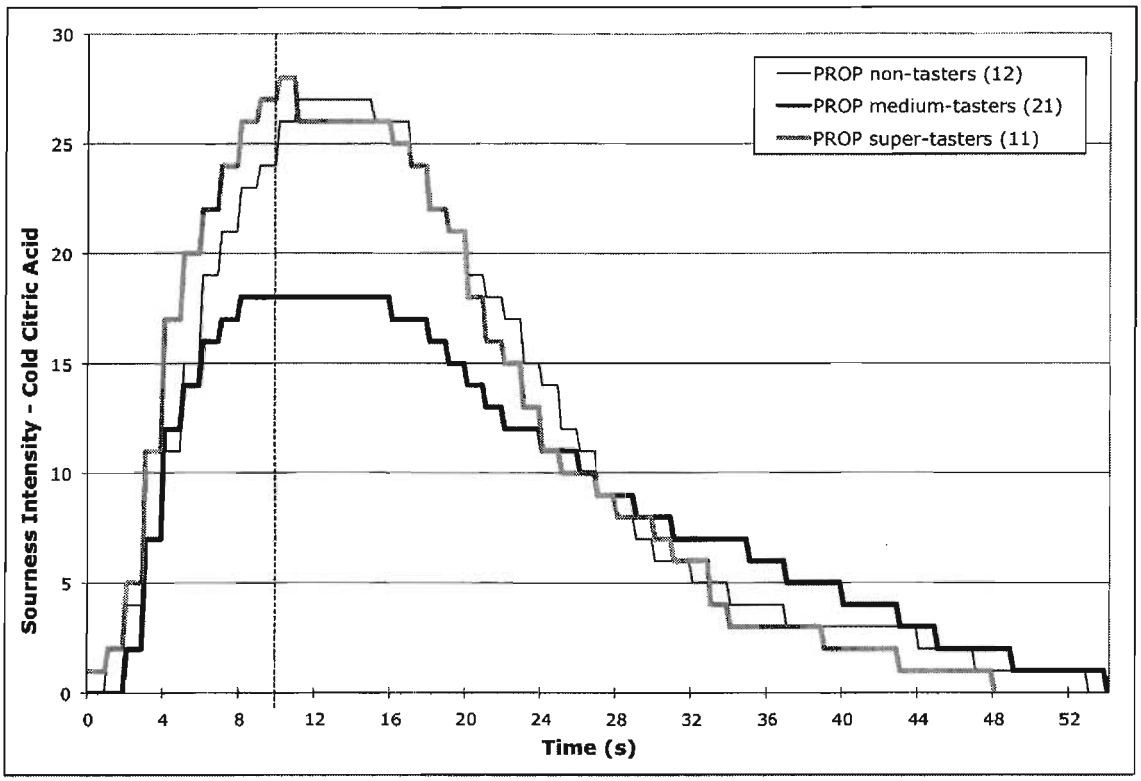
B)


PROP non-tasters (12)
PROP medium-tasters (21)
PROP super-tasters (11)
Bitterness Intensity - Cold Quinine
Time (s)

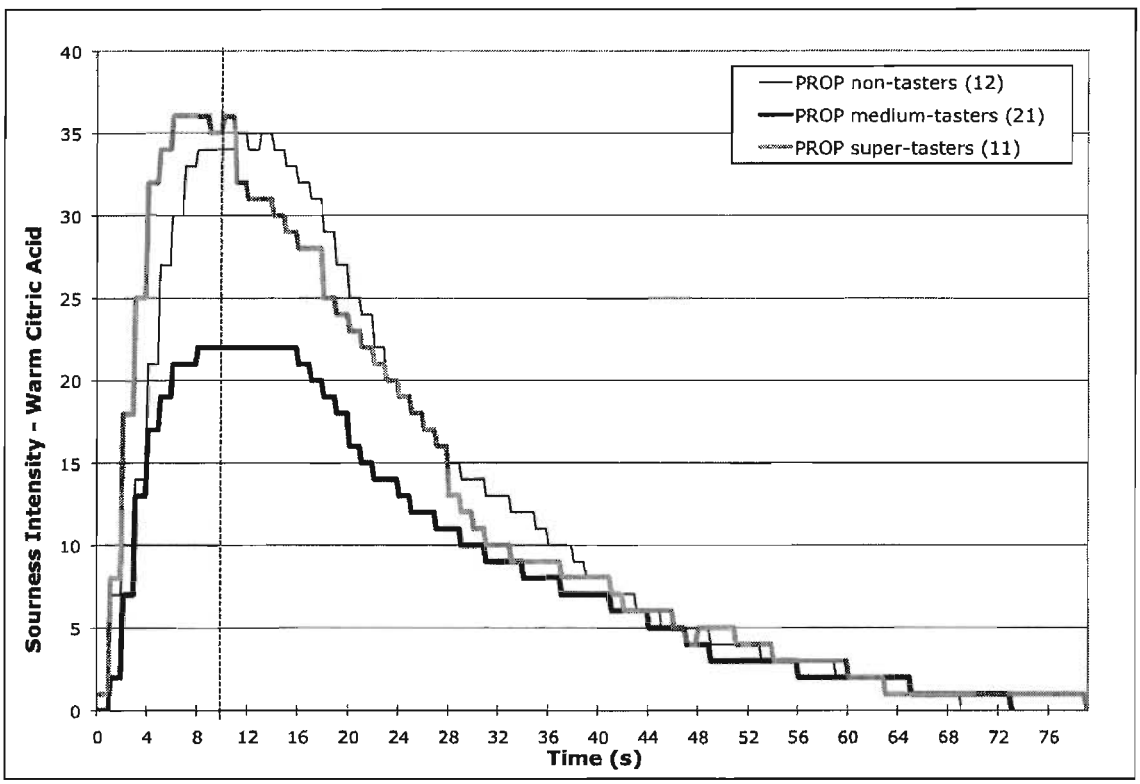
C)


PROP non-tasters (12)
PROP medium-tasters (21)
PROP super-tasters (11)
Bitterness Intensity - Warm Quinine
Time (s)

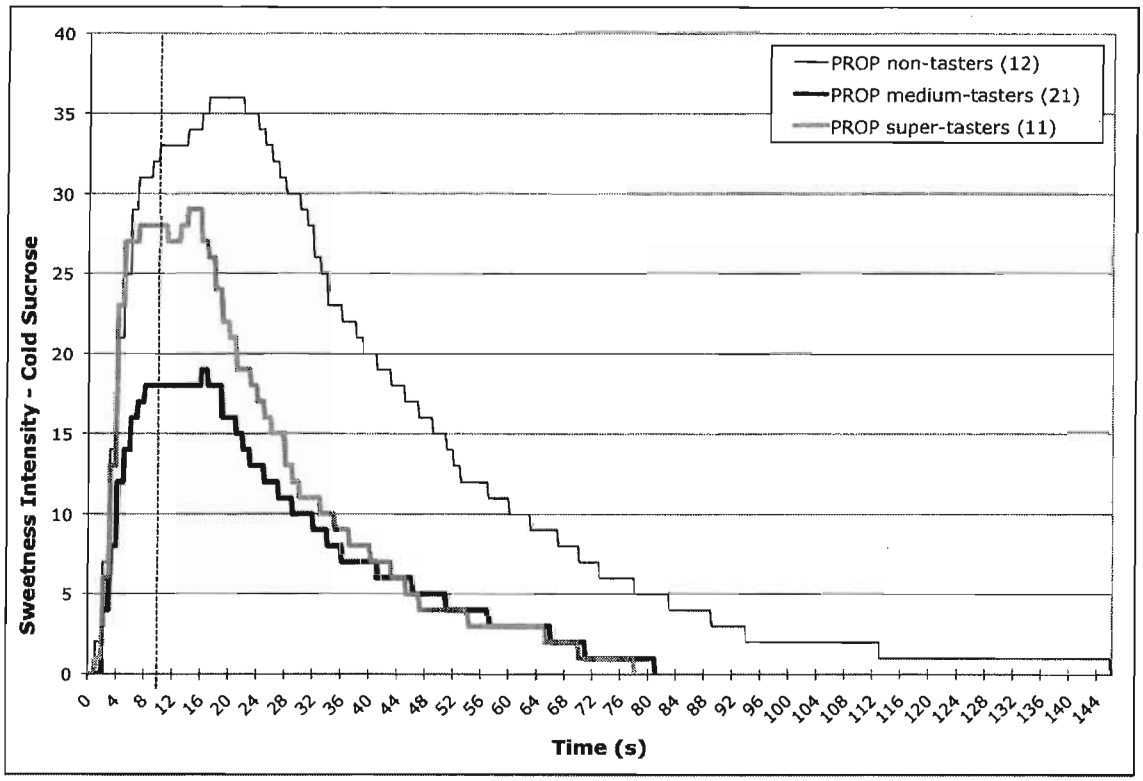
D)


PROP non-tasters (12)
PROP medium-tasters (21)
PROP super-tasters (11)
Sourness Intensity - Cold Citric Acid
Time (s)
0
5
10
15
20
25
30
0
4
8
12
16
20
24
28
32
36
40
44
48
52

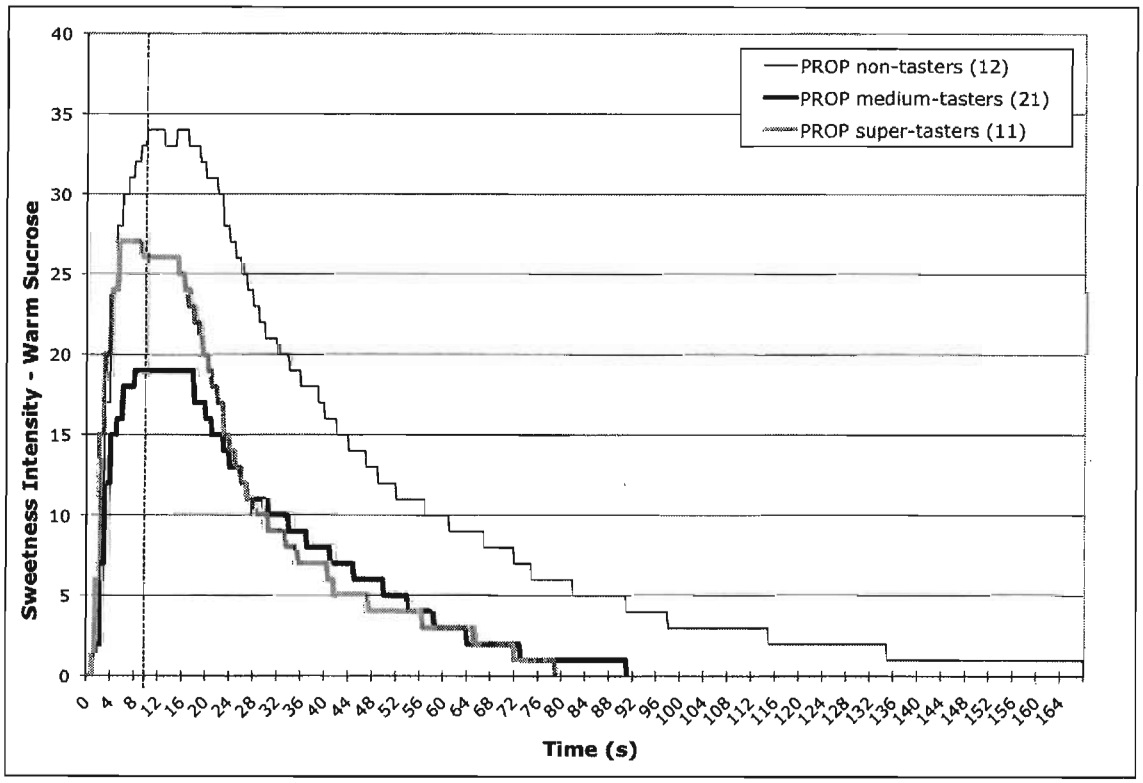
E)


PROP non-tasters (12)
PROP medium-tasters (21)
PROP super-tasters (11)
Sourness Intensity - Warm Citric Acid
Time (s)
0
5
10
15
20
25
30
35
40
0
4
8
12
16
20
24
28
32
36
40
44
48
52
56
60
64
68
72
76

F)


PROP non-tasters (12)
PROP medium-tasters (21)
PROP super-tasters (11)
Sweetness Intensity - Cold Sucrose
Time (s)

G)


PROP non-tasters (12)
PROP medium-tasters (21)
PROP super-tasters (11)
Sweetness Intensity - Warm Sucrose
Time (s)

H)

Figure 2. PROP non-taster, medium-taster, and super-taster averaged time-intensity curves for the perceived intensity of astringency from A) cold alum and B) warm alum, bitterness from C) cold quinine and D) warm quinine, sourness from E) cold citric acid and F) warm citric acid, and sweetness from G) cold sucrose, and H) warm sucrose. The vertical dashed-line at 10 seconds indicates sample expectoration.

(F(2,43)=5.71, P=0.007) significantly higher than pMTs. pNTs rated the IMax of warm sucrose significantly higher than pMTs (F(2,43)=4.82, P=0.013), and pNTs and pSTs rated cold sucrose IMax higher than pMTs (F(2,42)=7.9, P=0.001). pSTs had greater DAngs than pMTs for cold quinine (F(2,42)=5.79, P=0.006), cold sucrose (F(2,41)=4.922, P=0.012), and warm alum (F(2,43)=5.45, P=0.008). pNTs had a greater DAng than pMTs for DAng for warm citric acid (F(2,42)=3.65, P=0.035), and a greater DAng than either pMTs or pSTs for cold sucrose (F(2,43)=5.32, P=0.009). Cold sucrose DArea (F(2,41)=1.70, P=0.022) and AUC (F(2,41)=4.10, P=0.024) were significantly greater for pNT than pMT.

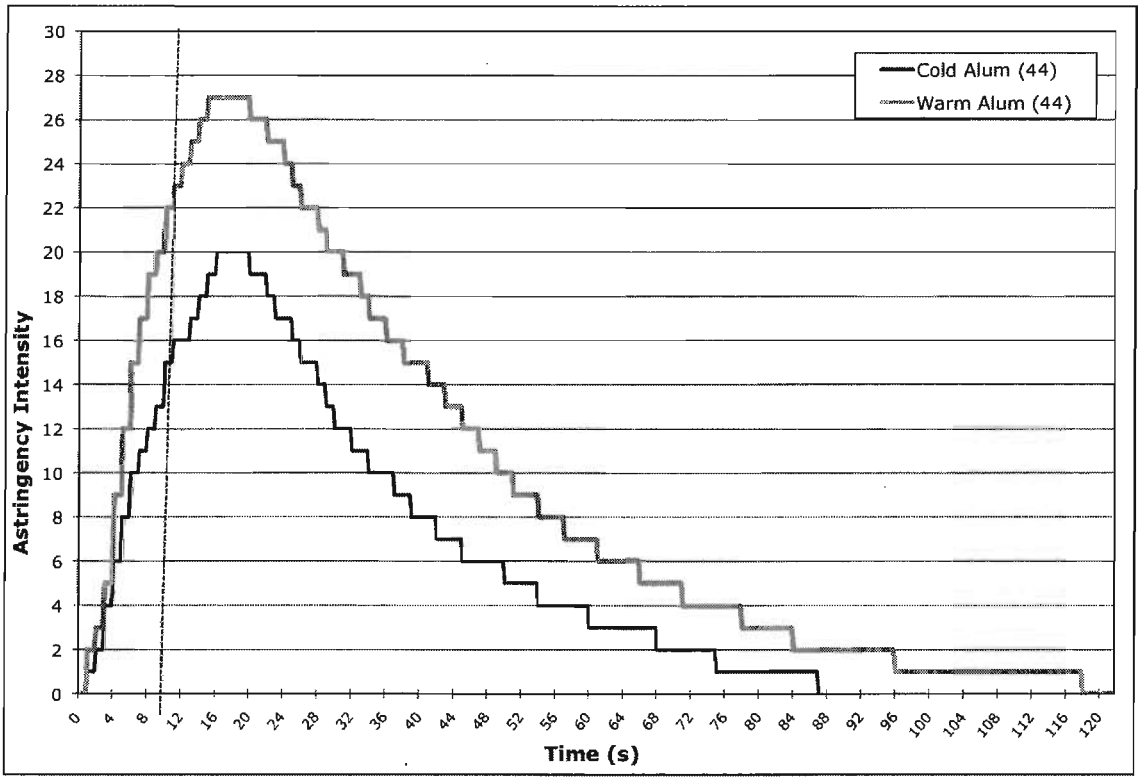
Temperature Effects Within PTS Groups

One-way ANOVA was employed to examine the influence of temperature on TI parameters in pNTs, pMTs, and pSTs separately. Alum IInt (F(1,23)=4.45, P=0.047), and citric acid IArea (F(1,22)=5.48, P=0.029) and AUC (F(1,22)=5.85, P=0.025) were significantly greater for warm than cold stimuli in pNTs. No significant differences were observed in TI parameters for bitter and sweet stimuli at the two temperatures for pNTs. Similarly, for pMTs no significant differences were observed for TI parameters of astringent, bitter, and sweet stimuli at the two temperatures. Cold citric acid IDelay (F(1,41)=4.29, P=0.045) was significantly longer than warm for pMTs. No significant differences were observed between cold and warm TI parameters for alum in pSTs. TMax (F(1,19)=9.49, P=0.006) and IArea (F(1,19)=9.22, P=0.007) were significantly longer and greater, respectively, for cold quinine than warm in pSTs, as was sucrose TMax (F(1,21)=4.55, P=0.046). Warm citric acid Dur (F(1,20)=7.00, P=0.016) was significantly longer than cold.

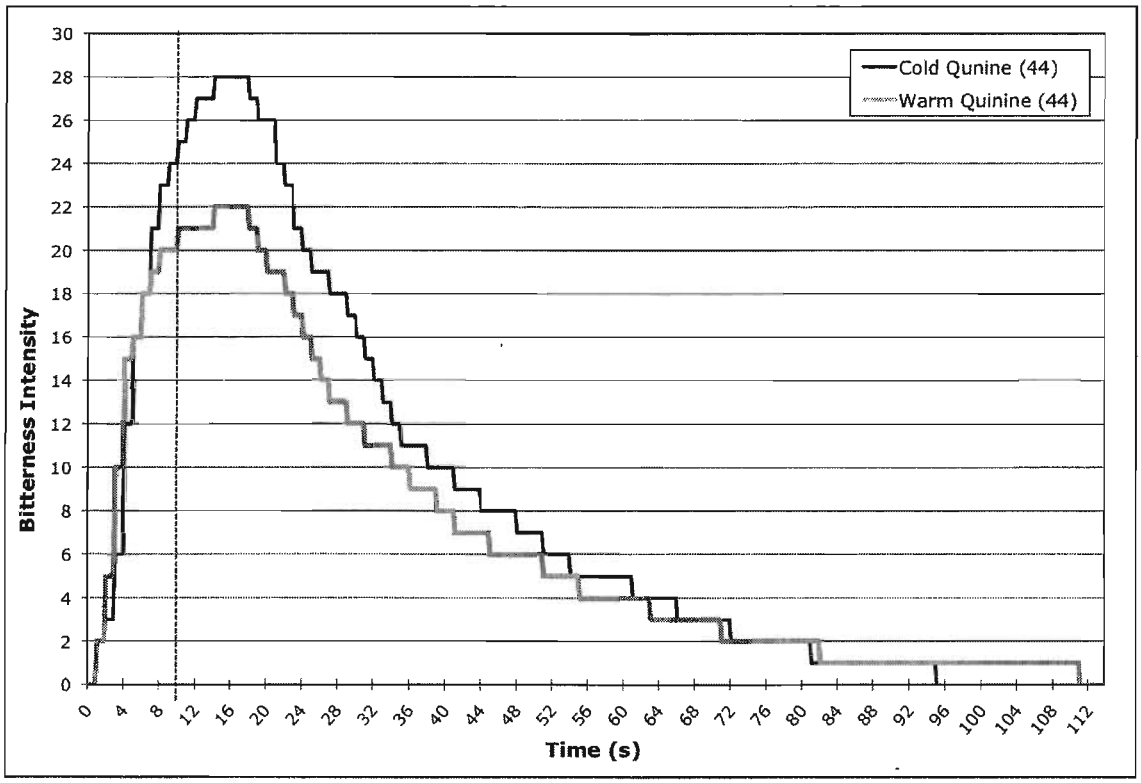
Temperature effects

To examine the influence of temperature on TI parameters, each of the four stimuli was presented at 5°C and 35°C and paired samples t-tests were performed. Averaged TI curves for each taste at each temperature are presented in Figure 3.

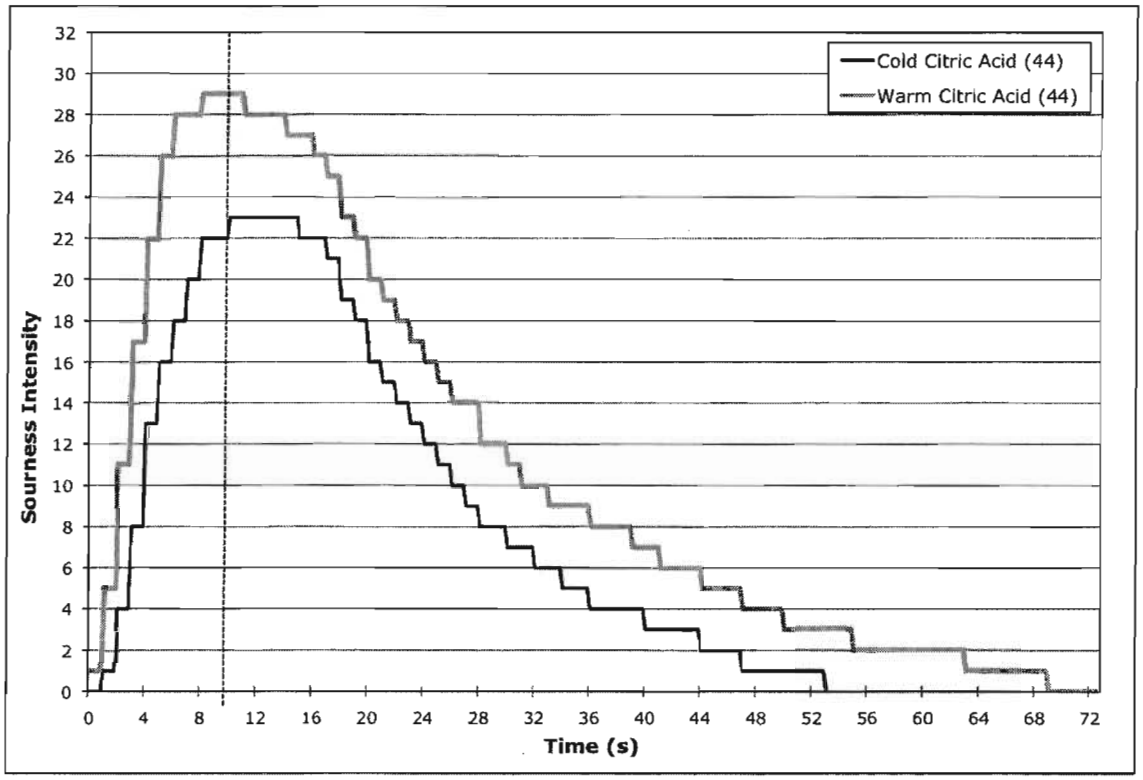
IArea (t(43)=4.03, P=2.23E-4), Imax (t(43)=4.67, P=3.06E-5), DArea (t(42)=3.57, P=9.15E-4), Dur (t(42)=4.74, P=2.46E-5), and AUC (t(42)=4.02, P=2.40E-4) were significantly greater for warm alum than cold. There was a trend for all


Cold Alum (44)
Warm Alum (44)
Astringency Intensity
Time (s)

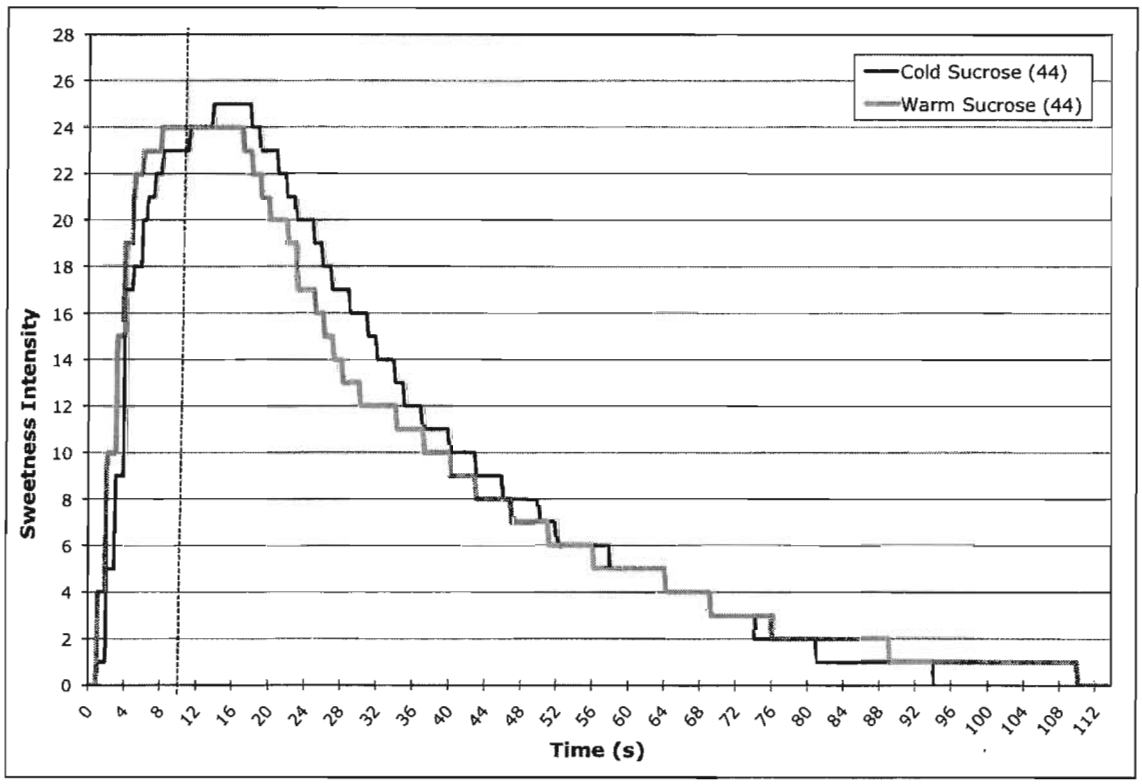
A)


Cold Qunine (44)
Warm Quinine (44)
Bitterness Intensity
Time (s)

B)


Cold Citric Acid (44)
Warm Citric Acid (44)
Sourness Intensity
Time (s)

C)


Cold Sucrose (44)
Warm Sucrose (44)
Sweetness Intensity
Time (s)

D)

Figure 3. Averaged time-intensity curves for the perceived intensity of A) astringency from warm and cold alum, B) bitterness from warm and cold quinine, C) sourness from warm and cold citric acid, and D) sweetness from warm and cold sucrose. The vertical dashed-line at 10 seconds indicates sample expectoration.

parameters, except Dur and IAng, to be greater for cold quinine than warm, which reached significance for IMax (t(43)=2.57, P=1.37E-2), and Dang (t(41)=2.04, P=4.82E-2). Warm citric acid IArea (t(43)=2.30, P=2.65E-2), IMax (t(43)=3.41, P=1.62E-2), Dur (t(43)=4.18, P=1.39E-4), DArea (t(43)=4.05, P=2.13E-4), and AUC (t(43)=4.20, P=1.34E-4) were significantly greater than cold citric acid, while the opposite was observed for IDelay (t(43)=2.76, P=8.43E-3), TMax (t(43)=2.76, 8.43E-3), and DAng (t(42)=2.15, P=3.74E-2). TMax (t(43)=3.13, P=3.11E-3) and IArea (t(43)=3.53, P=1.70E-3) were both significantly greater for cold sucrose than warm, while IAng (t(43)=3.17, P=2.79E-3) was greater for warm than cold.

Other Considerations

Using two-way ANOVA, no significant TTS*PTS interactions were observed (data not shown). Eta-squared values suggest that PTS generally accounts for more variation in TI parameters than TTS (Table 3).

Discussion

TTS

It was hypothesized that if TTs are more sensitive to thermal and taste stimuli than TnTs, then differences in temporal characteristics of orosensory stimuli at different temperatures may be observed. While there was a trend for TTs to produce higher maximum intensities than TnTs for most orosensory stimuli, it is unclear what this finding means. Green and colleagues have suggested that super-tasting results from a central nervous system gain mechanism in the afferent system mediating flavour perception (Green & George, 2004; Green et al., 2005). The globality of the trend for TTs to rate the perceived intensity of all orosensory stimuli presented, including the perceived intensity of thermal and tactile stimuli, which are mediated by the somatosensory system, suggest that this might be true for TTs. The only other TI parameter where a consistent trend for TTs was observed was in the rate of decline, which was greater for TTs than TnTs, suggesting that the intensity of orosensory stimuli drops off more rapidly for TTs than TnTs. To further delineate the TT phenomenon, whether TTs' ability to perceive a specific thermal taste predicts greater responsiveness

Table 3. Eta-squared values expressed as percents for PROP taster status (PTS) and thermal taster status (TTS) effects on time-intensity parameters of cold (c) and warm (w) orosensory sensations.

	Astringency		Bitterness		Sourness		Sweetness	
	TTS c/w	PTS c/w	TTS c/w	PTS c/w	TTS c/w	PTS c/w	TTS c/w	PTS c/w
TMax	2.8/0.7	4.2/2.2	5.9/0.0	1.2/23.5	1.5/0.0	9.7/8.5	1.6/3.0	5.5/10.9
IMax	0.9/2.7	14.8/11.5	0.1/0.0	16.8/7.4	4.5/1.8	5.7/18.5	2.3/4.3	24.9/13.7
Dur	1.8/0.0	0.3/1.6	0.1/0.1	7.6/8.5	0.1/0.0	8.8/1.3	2.5/5.3	15.1/6.2
AUC	0.2/0.5	2.0/4.4	0.1/0.1	5.0/2.5	1.3/0.2	1.9/6.3	0.3/2.4	16.2/5.6
IAng	0.7/1.7	9.0/10.9	2.1/0.0	16.3/22.9	1.2/1.5	20.6/18.4	0.1/0.3	23.7/8.1
IArea	0.1/0.0	1.7/3.8	2.8/1.2	3.9/12.8	3.6/2.7	0.9/3.9	0.6/7.0	7.3/5.0
DAng	0.4/0.2	8.7/22.4	0.7/3.3	19.5/3.8	0.3/0.1	8.8/14.2	0.4/0.3	19.7/6.2
DArea	0.2/0.6	2.3/4.1	0.0/0.0	6.7/1.8	0.4/0.0	4.1/5.8	0.3/0.9	17.1/4.1
IDelay	0.9/0.3	5.1/7.7	3.0/2.8	0.6/0.3	0.6/0.7	1.3/0.4	0.2/1.3	8.1/7.8
IInt	7.6/4.2	2.4/7.6	0.5/5.5	13.2/0.8	0.2/0.2	8.0/15.2	0.0/0.2	0.8/0.2

to temperature and the corresponding chemical tastant delivered together was investigated. The results described here do not support differences in the temperature sensitivity of taste transduction apparatus in TT sub-groups, further bolstering the central gain argument for the general orosensory responsiveness of TTs. However, these results must be interpreted with caution, as the number of subjects within each TT sub-group is low, and only one chemical tastant was employed as a representative of the prototypical tastes. We suggest that a larger study employing multiple tastants at a variety of concentrations and temperatures be undertaken to fully characterize TTs into sub-groups and examine the affect of the ability to thermal taste on the perception of chemical tastants and thermal stimuli.

PROP and PTS

The only relationship observed between PROP bitterness and a TI parameter was a negative correlation with the duration of sweetness from cold sucrose. Based on our previous results, we had expected PROP bitterness to correlate with maximum intensities, however, this was not the case. Within PTS groups (i.e., pSTs, pMTs, pNTs) TI parameters of stimuli at different temperatures corresponded to those observed in the cohort overall. Based on the extensive body of literature regarding PTS and the perception of prototypical tastants, it was expected that pSTs would perceive all stimuli with greater intensity than pMTs, who would perceive it with greater intensity than pNTs. Interestingly, however, this result was not observed. While pSTs did provide higher maximum intensity ratings than pNTs for some of the stimuli, pNT provided higher ratings for others, including bitterness from warm quinine, and sucrose sweetness at both temperatures.

An examination of the initial intensity, angle of incline, and the time to maximum intensity may provide an explanation for the unexpected results observed for these orosensory stimuli; in some cases, pSTs have a slightly greater initial intensity, rate of increase and a shorter time to maximum intensity. Using static rating methodologies, which are used in most studies investigating the relationship between PROP and responsiveness to orosensory stimuli, the perceived intensity of the sensation may have been rated sooner than the maximum intensity occurred. This would have

reflected a higher perceived intensity for pSTs and a lower perceived intensity for the other PTS groups, which may not have been truly reflective of their perceptions of the stimuli. A possible example of this can be observed for alum astringency for pNTs; Imm (Imm, 1997) noted that PROP non-tasters had a higher response to the perceived drying sensation from alum than tasters, however, the current study indicates that pSTs perceive both warm and cold alum with greater intensity than pNTs, but their maximum lags behind pNTs such that at a time of 5 s, which is a standard time for perceived intensity to be measures, pNTs would have had a higher perceived intensity. Further, the combined psychological effect of the slightly higher initial intensity, more rapid rate of increase, and shorter duration may have an influence on static quantifications of perceived intensity. The relationship between TI parameters and static measures is one that has not been described, but certainly deserves much more attention.

An interesting finding here is that pMTs maximum intensity ratings were lower than both pSTs and pNTs. Recent work by Hayes et al. (2008) suggests that PROP responsiveness is the culmination of multiple factors, and not just *Tas2R38* genotype. Hayes et al. (2008) observed that individuals homozygous for the AVI haplotype were not strictly pNTs, and those homozygous for the PAV haplotype were not strictly pSTs, as previously suggested; however, they also observed the expected positive relationship between PROP responsiveness and perceived intensity of prototypical tastants. This implies that PROP responsiveness is a predictor of responsiveness to taste stimuli, independent of TASR38 genotype. PTS categorization was performed here as described in Hayes et al. (2008), and the same concentration of PROP was used for categorization. The concentrations of quinine and sucrose differed between the two studies, which may have resulted in the discordant results; however, the citric acid concentration was well matched, suggesting concentration might not be the underlying reason for the difference. Hayes et al. (2008) used the gLMS for all intensity ratings, while here the gVAS was employed. The gVAS was used here as bench tests suggested it was easier to use for TI data collection, and, by ensuring the top anchor was outside of the modality of interest, it was expected to produce results comparable to those that would be obtained using the gLMS (L. Bartoshuk, personal communication). The lack of correlation between PROP bitterness intensity and the intensity of the orosensory

stimuli suggests that the lack of a PTS effect observed with ANOVA is not an artifact of the artificial categorization imposed by PTS grouping (Bajec & Pickering, 2008). This finding raises the question of whether super-tasting (i.e., elevated response to taste, retronasal, somatosensory, and chemesthetic stimuli; Hayes et al., 2008) is a temperature-dependent phenomenon, with some individuals being super-tasters at ambient temperature, some at warm and some at cold temperatures.

PTS groups were employed here to compare super-tasting by two different categorization criteria (i.e., PTS and TTS). As expected, no TTS*PTS interactions were observed on any of the TI parameters examined (Bajec & Pickering, 2008). Most Eta-squared values were larger for PTS than TTS, corroborating a past report suggesting that PTS has a greater affect on perceived intensity than TTS (Bajec & Pickering, 2008). The lack of association between TTS and PTS and the difference in Eta-squared values implies that the two indices of individual variation are independent, however, it does not rule out the possibility that 'super-tasting' (i.e., heightened response to orosensory stimuli) is a central rather than peripheral phenomenon, as has previously been suggested (Green et al., 2005).

Temperature

The influence of temperature (5°C and 35°C) on the temporal characteristics of astringency, bitterness, sourness, and sweetness was examined. The perceived intensity of astringency from warm alum increased at a greater rate, reached a higher maximum faster, decreased slower, and lasted longer than from cold alum. Although astringency elicited by alum is suggested to result from a different mechanism than that elicited by polyphenols (Peleg et al., 1998), it has been argued that alum is a suitable prototypical astringent based on its psychophysical characteristics (Lee & Lawless, 1991). Astringency elicited by polyphenols results from their ability to bind lubricative salivary proteins and precipitate them out of solution, leading to increased friction between the surfaces of the oral cavity and the stimulation of mechanoreceptors (reviewed in Bajec & Pickering, 2008). The protein-binding activity of alum is well documented (Trapp, 1983; Harris, 1996), suggesting it shares at least part of its mechanism of astringency elicitation with polyphenols. While the astringency of tannic

acid or catechin in water does not appear to differ in intensity at 7°C and 18°C (Valentova et al., 2002), the perceived astringency of cranberry juice decreases with decreasing temperature (Peleg & Noble, 1999). In model protein systems, polyphenol binding to proteins appears to be stronger at higher temperatures (Artz et al., 1987; Hofmann et al., 2006). This suggests that the results described here for warm versus cold alum may be due to the formation of stronger, more enduring bonds between alum and salivary proteins leading to greater perceived astringency of a longer duration.

The perceived bitterness from cold and warm quinine increased at the same rate, however, the time to maximum and maximum intensity were greater for cold quinine. Additionally, the rate of bitterness decline was greater for cold quinine. These results suggest that, although it takes longer to reach its maximum and it doesn't last as long, the intensity of bitterness from cold quinine reaches a greater maximum than cold. Data for caffeine indicates that bitterness elicited by warm solutions (36°C) is greater than cool (20°C; Green & Frankmann, 1987). While evidence suggests that both caffeine and quinine function through the activation of taste receptors (Zhang et al., 2003; Damak et al., 2006; Lee et al., 2009), both are also known to have other activities (Rosenzweig et al., 1999; Peri et al., 2000; Mao et al., 2007). Owing to its amphiphilicity, quinine is capable of receptor-independent G-protein activation (Naim et al., 1994), and has a demonstrated inhibitory action on K^+ conductance (Tsunenari et al., 1996). These differences in activity, and/or others as yet unknown, may account for the differences in temperature-dependence observed between the two compounds. Quinine thresholds appear to have a positive relationship with temperature (Paulus & Reisch, 1979); however, the influence of threshold on suprathreshold measures is difficult to interpret.

Sourness from warm citric acid reached a greater maximum intensity at a greater rate than cold citric acid. Additionally, sourness from warm citric acid lasted longer and declined more slowly than cold citric acid. Given past reports, an affect of temperature on citric acid sourness was not expected (Green & Frankmann, 1987), however, the results of Moskowitz (1973) suggest that the intensity of citric acid at 35°C is greater than at 25°C for all concentrations. Interestingly, the sourness of cheddar cheese is positively associated with temperature, but in wine the affect of serving temperature on

perceived sourness appears to be dependent on the individual (Drake et al., 2005; Ross & Weller, 2008).

Perhaps the most interesting and unexpected effect of temperature on perceived intensity of taste was that observed for sweetness from sucrose. Although the sweetness perceived from warm sucrose increased more rapidly, and lasted longer than sweetness from cold sucrose, its maximum was not significantly different. This finding contradicts past reports where warm sucrose was rated higher in intensity than cold sucrose (Green & Frankmann, 1987; Green & Frankmann, 1988; Bartoshuk et al., 1982; Calvino, 1986); however, others have reported the temperature-independence of sweetness intensity, specifically for dextrose and fructose independently and in combination, as well as for sucrose (Schiffman et al., 2000; Stone et al., 1969). Using similar sucrose concentrations (219 mM and 292 mM) to that employed here, Schiffman et al. (2000) reported no difference in the perceived intensity of sucrose sweetness, and in fact, a cold solution (6°C) was rated slightly more intense at the lower concentration. The lack of a temperature affect on the perception of sweetness seems to contradict the idea that TRPM5 acts as a coincidence detector of thermal and depolarizing stimuli, however, an investigation using more and a larger range of temperatures and concentrations of sucrose would have to be undertaken to substantiate such a conclusion.

Other Considerations

Methodological differences offer a simple explanation for the discrepancies between the current results and previous reports. A major difference between the current study and others is the maintenance of the buccal cavity at ~37°C (Green & Frankmann, 1987; Moskowitz, 1973; Larson-Powers & Pangborn, 1978). It should be noted that past studies examining temperature-taste interactions did not all have the same objectives as the current study. For example, Green & Frankmann (1988) were examining whether the tongue temperature or solution temperature is of greater importance, while Moskowitz (1973) focus was the constant of the psychophysical function. When considering the current and past work examining the affects of temperature on taste, the protocol of temperature delivery must be considered, and results and implications interpreted accordingly. Tongue temperature was not controlled

here in an attempt to maintain ecological validity, as during normal eating behaviour the tongue is not maintained at a specific temperature, but rather changes with each bite or sip and returns back to baseline.

Other methodological differences between studies examining the effects of temperature on taste must also be noted. Larson-Powers & Pangborn (1978) employed a two-sip and spit paradigm that differs from that used here and could influence their results, given that adaptation occurs with taste (reviewed in Bajec & Pickering, 2008). Given the relationship between the perceived intensities of tastants, use of an intensity scale that has as its top anchor the 'strongest sensation ever experienced' in all sense modalities, such as the gVAS, removes the relativity of ratings, resulting in independent ratings of intensity for each tastant (Bartoshuk et al., 2002). The duration that the tastant solution was held in the mouth and the time after sampling that the intensity rating was provided also differed between the studies discussed here. While many indicate a rinse duration of 4-5 seconds, some provide no indication of the rinse duration (e.g., Green & Frankmann, 1987; Green & Frankmann, 1988; Bartoshuk et al., 1982). Further, Mosowitz (1973) had subjects rate the intensity of tastant solutions immediately upon putting them in the mouth. It is quite obvious from the results presented here and in other TI studies that the time-course of perceived intensity changes rapidly with some tastants, and that the initial intensity is normally not the maximum. As such, the tastant being examined and the desired metric must be taken into account when an experimenter is determining both the duration that stimuli are to be held in the mouth and lag time before the intensity rating is made. Indeed, here maximum intensities were often reported after expectoration.

Conclusion

The effect of temperature on the perception of orosensory stimuli using TI methodology was examined. The use of TI methodology over static intensity collection measures provides additional dimensions to the investigation of taste perception, and allows for a more comprehensive understanding of the perception of orosensory stimuli. Further, the effect of TTS and PTS, and their interaction, on the relationship between temperature and TI parameters was examined. Temperature appears to influence the perception of

astringency, bitterness, sourness, but not sweetness. As expected, a trend of TTs reporting higher maximum intensities was observed. A preliminary examination of TT sub-groups did not uncover differences in their perception of cold and warm stimuli, however, a larger study of TT sub-groups is required to draw a meaningful conclusion. Unexpectedly, PROP bitterness was not associated with the maximum perceived intensity of the orosensory stimuli examined, and the anticipated PTS effect was not observed. However, some differences between PTS groups in their perception of orosensory stimuli over time were found. In accordance with previous reports no interaction was found between PTS and TTS, and, using Eta squared (η^2), PTS was observed to exert more of an affect on TI parameters than TTS.

The use of TI methodology should be considered for all investigations of orosensory psychophysics and individual variation in order to determine true maxima and draw meaningful comparisons between individuals. Further, it would be of great benefit to examine the relationship between static intensity measures and the maximum intensity metric determined using TI methodology to identify the optimal time to record this important parameter of perception.

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CHAPTER 5: ASSOCIATION OF THERMAL TASTE, PROP RESPONSIVENESS AND GENDER WITH LIKING AND CONSUMPTION OF ALCOHOLIC BEVERAGES

Introduction

While humans perceive bitter, sour, sweet, and salty tastes from ethanol, sweetness and bitterness are the dominant gustatory sensations elicited by ethanol (Mattes and DiMeglio, 2001; Scinska et al., 2000). In rodents and humans, sweetness is of particular importance to ethanol consumption (Blizard, 2007; Blednov et al., 2008; Kampov-Polevoy et al., 1999). While some studies suggest that sweet-liking is associated with greater alcohol intake and a genetic risk for alcoholism (Kampov-Polevoy et al., 2003; Kampov-Polevoy et al., 2004), others have not found a relationship between the two (Bogucka-Bonikowska et al., 2001; Kranzler et al., 2001; Scinska et al., 2001). For some beverages, a relationship between bitterness intensity and alcohol intake has been demonstrated, with lower perceived bitterness associated with increased intake (Guinard et al., 1996; Lanier et al., 2005). 6-n-propylthiouracil (PROP) bitterness, a well-established index of individual variation in taste perception, has also been suggested to associate with alcohol intake.

PROP responsiveness is typically expressed categorically as PROP taster status (PTS), which consists of three groups: PROP super-tasters (pSTs), PROP medium-tasters (pMTs), and PROP non-tasters (pNTs) (Bartoshuk, 1993), with pSTs being most responsive to the bitterness of PROP, pNTs least responsive, and pMTs presenting intermediate responsiveness. While other genes are also thought to be involved in the perceived bitterness of PROP (Reed et al., 1999; Drayna et al., 2003; Hayes et al., 2008), molecular data indicate that the *TAS2R38* gene encodes two major forms of the PROP receptor, PAV and AVI; typically, those individuals that carry two PAV alleles are very responsive to PROP, those with two AVI alleles are minimally or non-responsive, and those with one PAV allele and one AVI allele demonstrate intermediate responsiveness (Duffy et al. 2004a; Hayes et al., 2008). The physiological basis for the differences in oral perception between pSTs, pMTs, and pNTs appears to be fungiform papillae (FP) density and taste pore number, with pSTs having a significantly greater

number of FP and taste pores on the anterior surface of the tongue than pMTs and pNTs (Bartoshuk et al., 1994, Tepper and Nurse, 1997; Bajec and Pickering, 2008). In addition to bitterness, the irritation from ethanol (Bartoshuk et al., 1994; Karrer and Bartoshuk, 1991; Bartoshuk et al., 2000; Duffy et al., 2004b) and the tactile sensation of astringency (Pickering et al., 2006; Pickering and Robert, 2006), which are important contributors to the overall flavour of alcoholic beverages, are also perceived with greater intensity by those who perceive PROP bitterness with greater intensity (Duffy et al., 2004a; Bajec and Pickering, 2008). Additionally, individuals with a greater density of FP, which are also innervated by trigeminal fibers (Farbman and Hellekant, 1978; Whitehead et al., 1985), have been reported to perceive alcohol burn with greater intensity (Duffy et al., 2004a; Duffy et al., 2004b).

Studies examining an association between PROP tasting and alcohol intake and/or alcoholism have returned conflicting results. In an examination of beer consumption, Guinard et al. (1996) found that a high-consumption group contained a greater number of pNTs and a lesser number of pSTs compared to a low-consumption group. Intranuovo and Powers (1998) reported that pSTs consumed less beer when they first started drinking compared to pNTs, however, there were no differences between the two groups in their current consumptive behaviours. Interestingly, DiCarlo and Powers (DiCarlo and Powers, 1998) found that individuals with alcoholism in their families were more likely to be pNTs, while those that had both alcoholism and depression in their families were pSTs, suggesting PROP might function as a genetic marker in Type I and Type II alcoholism. Using phenylthiocarbamide (PTC), Driscoll et al. (2006) found that male pSTs had fewer problems with alcohol and less family history of alcoholism than male pNTs, while the relationship was reversed with females. Based on a higher proportion of pNTs among children of alcoholics, Pelchat and Danowski (1992) concluded that there is a genetic association between PROP-tasting and alcoholism. On the contrary, Kranzler and colleagues (Kranzler et al., 1996; Kranzler et al., 1998) found no association between parental alcoholism and PTS in offspring. Mattes and DiMeglio (2001) also found no association between PTC-tasting and alcohol intake. Duffy et al. (2004b) suggest that the inconsistencies reported in

PROP effects on alcohol intake could be the result of issues related to the different measurement techniques used for PTC/PROP-tasting and categorization.

An inverse relationship between PROP intensity scores and alcohol intake supports the hypothesis that pSTs are protected from alcoholism because of their increased responsiveness to bitterness and the burn from ethanol (Duffy et al., 2004b; Intranuovo and Powers, 1998; Driscoll et al., 2006). Concomitantly, pNTs are generally hypothesized to be more susceptible to alcohol misuse because of their decreased responsiveness to bitterants and ethanol (Duffy et al., 2004b; Intranuovo and Powers, 1998; Driscoll et al., 2006). Recent work from Duffy and co-workers, employing PROP bitterness as a continuous measure rather than a categorical variable, provides evidence of a link between both PROP bitterness and the *TAS2R38* genotype, and alcohol intake. Individuals who perceive PROP as more bitter consume fewer alcoholic beverages per year than those that perceive PROP as less bitter (Duffy et al., 2004a; Duffy et al., 2004b). Similarly, PAV/PAV homozygotes consume fewer alcoholic beverages per year than either AVI/PAV or AVI/AVI individuals, and AVI/PAV heterozygotes consume less than AVI/AVI homozygotes (Duffy et al., 2004a). For scotch, the perceived bitterness and sweetness of the sample predicted total alcohol intake, with the bitterness and sweetness of the scotch predicted by PROP bitterness (Lanier et al., 2005). While PROP bitterness also predicted the perceived bitterness of sampled beer, which in-turn predicted liking, total alcohol intake was predicted by beer liking but not by bitterness, with those who liked the beer sample more consuming more alcohol (Lanier et al., 2005). These findings corroborate a previous study, which found that pSTs rated the bitterness of beer as more intense than pMTs or pNTs, and that higher beer bitterness ratings were correlated with greater disliking for the beer sample (Intranuovo and Powers, 1998).

A greater proportion of pSTs are females (Bartoshuk et al., 1994), as such, it might be expected that females would like alcoholic beverages less and have a lower intake of alcoholic beverages than males. Indeed, females do rate the intensity and their dislike of alcohol burn higher than males; however, this finding could not be attributed to differences in PROP tasting across genders (Lanier et al., 2005). Interestingly, females appear to have a preference for wine, and males prefer beer (Barefoot et al.,

2002; Klatsky et al., 1983; Tjønneland et al., 1999; Grønbæk et al., 2000). While some studies have found differences between males and females in their total intake of alcoholic beverages (Duffy et al., 2004a; Barefoot et al., 2002; Klatsky et al., 1983; Wilsnack et al., 2000), others have not (Duffy et al., 2004b), and some have found that gender is a relatively minor contributor when other variables are considered (Becker and Kronus, 1977).

Recently, Green and co-workers identified a new marker of individual variation in oral sensation: thermal taste (Cruz and Green, 2000). When a small area of the tongue is heated and/or cooled, thermal tasters (TTs), who constitute approximately 20-50% of the population sampled, perceive a phantom taste (Bajec and Pickering, 2008; Green and George, 2004). Evidence from *Trpm5* knockout mice strongly suggests that TRPM5, a TRP superfamily cation channel with a role in the transduction of umami, sweet and bitter tastes (Zhang et al., 2003), plays a role in thermal taste (Talavera et al., 2005), presenting the possibility that this source of individual variation is under genetic control. Not only do TTs perceive a taste sensation from thermal stimuli, they also rate salt, citric acid, quinine, PROP, and monosodium glutamate applied to the tongue tip, as well as whole-mouth rinses of sucrose, citric acid, and PROP, as significantly more intense than thermal non-tasters (TnTs) (Bajec and Pickering, 2008; Green and George, 2004). Interestingly, ratings of burning, stinging and prickling produced by capsaicin and menthol do not differ between TTs and TnTs (Green et al., 2005); however, TTs perceive low and high levels of astringency with greater intensity than TnTs (Bajec and Pickering, 2008). TTs and TnTs do not differ in FP density, and PTS and thermal taster status (TTS) do not interact for intensity ratings of orosensory stimuli (Bajec and Pickering, 2008). PTS and TTS appear to function via independent mechanisms (Bajec and Pickering, 2008), which suggests TTS may be a non-PROP dependent way to identify supertasters.

Although alcoholic beverage liking affects consumption behaviours (Lanier et al., 2005), and recent work strongly suggests that liking is a valuable measure of food intake as it lacks the reporting and recall biases of self-reported intake (Duffy et al., 2009), few studies have examined the relationship between liking of alcoholic beverages and intake. The main aim of the current work was to examine the potential

influence of PTS and TTS on self-reported alcoholic beverage liking and intake. We also sought to investigate potential associations between the perceived intensities of prototypical orosensory stimuli (sweet, sour, bitter, salty, astringent, metallic), FP density, gender, and age with alcohol liking and intake. Additionally, the relationship between liking and self-reported intake was examined.

Methods

Subjects

132 subjects were recruited from the student, staff, and faculty populations of Brock University, and from the local community. Incentive was provided in the form of a monetary prize or credit toward a 1st year university Psychology course. 9 subjects were removed from the dataset for the current analysis as they reported abstinence from alcoholic beverage consumption. The final cohort consisted of 80 females and 43 males with a mean age of 31 years+/-11SD (range: 18 to 68). To establish ethnic origin, the Census Canada "Ethnic Origin User Guide" (Statistics Canada, 2001) was employed. Accordingly, and herein, the term Caucasian refers to those that reported 'White' as their ethnicity, and non-Caucasian refers to the group of subjects composed of all other ethnicities. 102 subjects were Caucasian (34 males), and the remaining 21 were non-Caucasian (9 males). 14 subjects reported that they smoked: 3 females, and 11 males. The Brock University Research Ethics Board approved all procedures, and written consent was obtained from all subjects.

Scale

Paper versions of the general Labeled Magnitude Scale (gLMS) were used to collect all psychophysical data (Bartoshuk et al., 2002; Bartoshuk et al., 2004). Subjects received verbal and written instructions that the top of the scale represented the most intense sensation in any modality that they could ever imagine experiencing, and were told to think of experiences from a variety of different modalities to assist in understanding the general nature of the scale (Bartoshuk et al., 2002). In order to familiarize subjects with the gLMS, and facilitate correct scale use, they were asked to rate the intensities of 5 remembered sensations: sourness of a lemon, pain from biting your tongue, coolness of

an ice-cold beverage, burning sensation from eating a whole hot pepper, brightness of the sun when looking directly at it (Green and Hayes, 2004; Porubcan and Vickers, 2005).

Prototypical Tastants, Astringents, Metallic Stimuli

Aqueous samples of oral stimuli were presented at room temperature as exemplars of the different taste and non-taste oral qualities and to obtain ratings of perceived intensity. Subjects evaluated the intensity of low and high levels of astringent (0.73 mM and 14.6 mM alum; Sigma-Aldrich, MO, USA), salt (10.5 g/L NaCl; Windsor, QC, Canada), sweet (147.2 g/L sucrose; Lantic Sugar Ltd., QC, Canada), sour (4.47 mM tartaric acid; Carl Roth KG, distributed by Atomergic Chemetals Corp., NY, USA), bitter (0.02 g/L quinine sulfate; Novopharm, ON, Canada), and metallic (0.3 mM and 3 mM iron (II) sulfate; J.T. Baker; NJ, USA) stimuli presented in random order (Bajec and Pickering, 2008).

6-n-propylthiouracil (PROP)

Subjects rinsed with a 20 ml volume of 0.32 mM 6-n-propylthiouracil (PROP; MP Biomedicals; OH, USA) solution, or as much as physically possible, for 10 s, expectorated, and waited for the bitterness intensity to peak (on average 10-15 s) before providing a rating (Bajec and Pickering, 2008).

Fungiform Papillae (FP) Density

Subjects' tongues, dyed with blue food colouring (Horton Spice Mills Ltd., ON, Canada), were photographed using a Canon Powershot S3 IS 6.0 megapixel camera in super macro mode mounted on a mini-tripod. Images were imported into and manipulated using Photoshop (CS2 9.0.2; Adobe; ON, Canada) on an iMac computer (Apple; CA, USA). All FP within a 0.6 cm diameter circle on each side of the anterior dorsal midline of the tongue were counted, averaged, and the FP density (FP/cm^2) calculated (Bajec and Pickering, 2008).

Thermal Taste (TT)

A 64 mm^2 computer-controlled Peltier device with a thermocouple feedback attached to a toothbrush-sized water-circulated heat sink (i.e., thermode) was applied to the S's extended tongue by the researcher. Subjects were instructed to rate the intensity of all oral sensations, including temperature that they perceived in each trial. Three locations on the edge of the tongue were stimulated discretely and in order: the most anterior tip, and approximately 1 cm to the right and then the left of the midline. Warming trials started at 35°C, cooled to 15°C, and re-warmed to 40°C (held for 1 s). The start temperature for cooling trials was 35°C, followed by cooling to 5°C (held for 10 s). Warming trials preceded cooling trials at each location to avoid possible adaptation from the intense, sustained cold stimulation (Green and George, 2004), and all warming trials (tip, right, left) were performed before all cooling trials (Bajec and Pickering, 2008).

Alcohol Liking and Consumption

Between the measurement of psychophysical and physiological variables, subjects completed questionnaires including alcohol liking and consumption measures. Liking of alcoholic beverages was determined by subjects rating their liking of a list of alcoholic beverages presented with examples on a 7-point Likert scale (Lawless and Heymann, 1998) ranging from 'like extremely' to 'dislike extremely'. Subjects could also indicate allergy, lack of exposure to the beverage, and lack of knowledge of the beverages in lieu of providing a liking rating. A measure of liking of alcoholic beverage categories, which included *beer* (ale, pale ale, lager, lambic beer, light beer, mild/brown ale, pilsner, strong beer, stout/porter, wheat beer), *wine* and the sub-groups *sweet wine* (dessert/ice wine, fruit wine, sweet red wine, rosé/blush, sweet white wine, sweet sparkling wine) and *dry wine* (red dry, white dry wine, dry sparkling wine), *spirits* and the sub-groups *unmixed spirits* (bitters, bourbon, brandy, gin, rum, rye, scotch, mixed bitter/sour/spicy, mixed sweet shots, tequila, vodka) and *mixed spirits* (mixed gin, mixed rum, mixed rye, mixed tequila, mixed vodka), and *other* (cider; port, sherry, wine

cooler, rum cooler, cream liqueurs, clear liqueurs) was calculated as a weighted average of all liking scores from that type of beverage. Additionally, a measure of *overall alcoholic beverage* liking was calculated as a weighted average of all liking scores from all beverages.

Alcohol consumption frequency (F) was measured by subjects indicating how many days per month they consumed beverages in each category (i.e., BEER, SPIRITS, RED WINE, WHITE WINE, OTHER). Subjects were instructed that the category OTHER could be used to indicate any beverages that were not readily identifiable as one of the types noted above. To determine how many beverages subjects consumed per drinking occasion (Q), they were asked how many drinks (standard drink size indicated as: 355 ml (12 oz.) bottle of beer; 177 ml (6 oz.) glass of wine; 44 ml (1.5 oz.) spirit) they consumed on days when they drank. To determine how many drinks of each beverage type subjects consumed per month (d/m), the frequency of consumption was multiplied by the number of drinks consumed per drinking occasion. Total drinks consumed per month (TOTAL d/m) was calculated as the sum of monthly consumption of all beverage types.

Data Treatment

In order to compare perceived intensities of psychophysical stimuli across individuals, data were rescaled relative to a non-taste sensation (Bartoshuk et al., 2002). The remembered intensity of "brightness of the sun when looking directly at it" was used to standardize the data (Bartoshuk et al. 2002; Porubcan and Vickers, 2005). Each subject's brightness rating was divided by the group average for this remembered sensation, creating an individualized normalization factor by which ratings for taste and non-taste oral sensations, including PROP and temperature from thermal stimulation, were divided.

PROP Taster Status (PTS) and Thermal Taster Status (TTS) Categorization

Duplicate PROP intensity ratings were averaged and PTS groups were defined as: pNTs<10.9 mm; pMTs 10.9-61.5 mm; and pSTs >61.5 mm (Porubcan and Vickers, 2005).

In order to maintain the position of "weak" on the gLMS, which was used to categorize individuals, thermal taste data were not normalized. TTs were defined as those that reported the same taste sensation, rated above weak, at the same location and temperature in both replicates (Green and George, 2004). Those that did not perceive any taste sensations in any trial were defined as TnTs.

Statistical Analysis

All analyses were performed with SPSS 16 (SPSS Inc., IL, USA). Univariate outliers were defined as having a standardized Z-score >/= |3.29| (Tabachnick and Fidell, 2006). Skewed variables, defined as those having a skew or kurtosis of greater than +/-2, were transformed (square root (sqrt) or log) to improve distribution normality for all statistical analyses (Tabachnick and Fidell, 2006). Two-way ANOVA examining the effects and interactions of PTS and TTS with gender was performed on liking scores of general alcoholic beverage types and individual alcoholic beverages. Where gender effects and interactions were not significant, one-way ANOVA was employed to examine effects of PTS and TTS independently. Where applicable, Tukey's HSD was used as the mean separation test following a significant one- or two-way ANOVA. The perceived intensity of PROP, taste and non-taste oral stimuli, and FP density were treated as continuous variables to allow for the examination of their possible association with individual and group alcoholic beverage liking scores and consumption parameters. Additionally, PROP intensity, FP density, age, and gender were used in a standard multiple regression to predict alcoholic beverage liking and consumption data (Duffy et al., 2004a). A similar regression analysis was employed to predict alcoholic beverage liking and consumption from TTS, FP density, age, and gender. Multivariate outliers were defined using the Mahalanobis distance criteria (χ^2 critical with $p<0.0001$; degrees of freedom equal to the number of independent variables (Tabachnick and Fidell, 2006).

Results

PTS categorization yielded 35 pNTs (12 males, 6 non-Caucasian, 6 smokers), 62 pMTs (24 males, 14 non-Caucasian, 7 smokers), and 26 pSTs (7 males, 1 non-Caucasian, 1

smoker). TTS categorization yielded 23 TTs (9 males, 4 non-Caucasian, 0 smokers), and 48 TnTs (14 males, 7 non-Caucasian, 6 smokers). 52 subjects could not be categorized for TTS. Analyses of psychophysical and physiological measures examined in this subject cohort (N+3) are presented in (Bajec and Pickering, 2008).

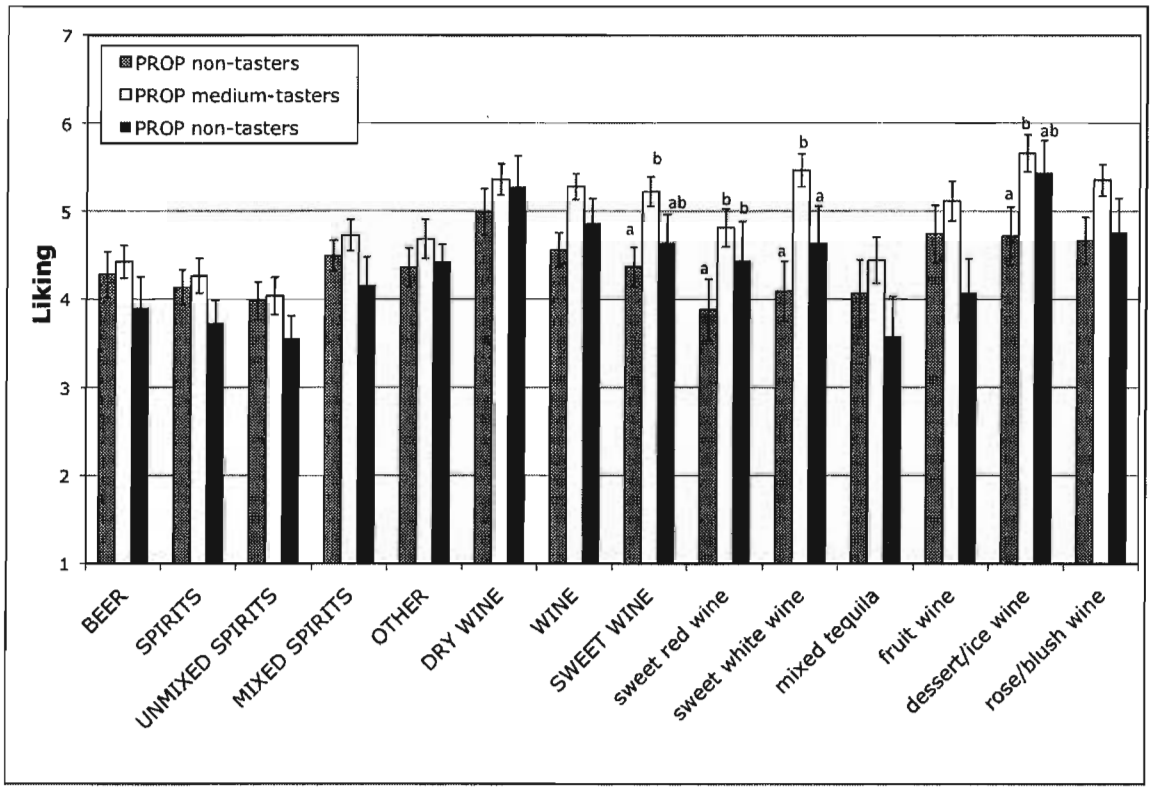
Alcoholic Beverage Liking

Liking of alcoholic beverage types and individual alcoholic beverages was examined and is reported below. Means and Tukey's HSD results for significant ($p < 0.05$) and near significant ($p < 0.1$) PTS, TTS, and gender effects on liking are summarized in Figures 1A), 1B), and 1C), respectively.

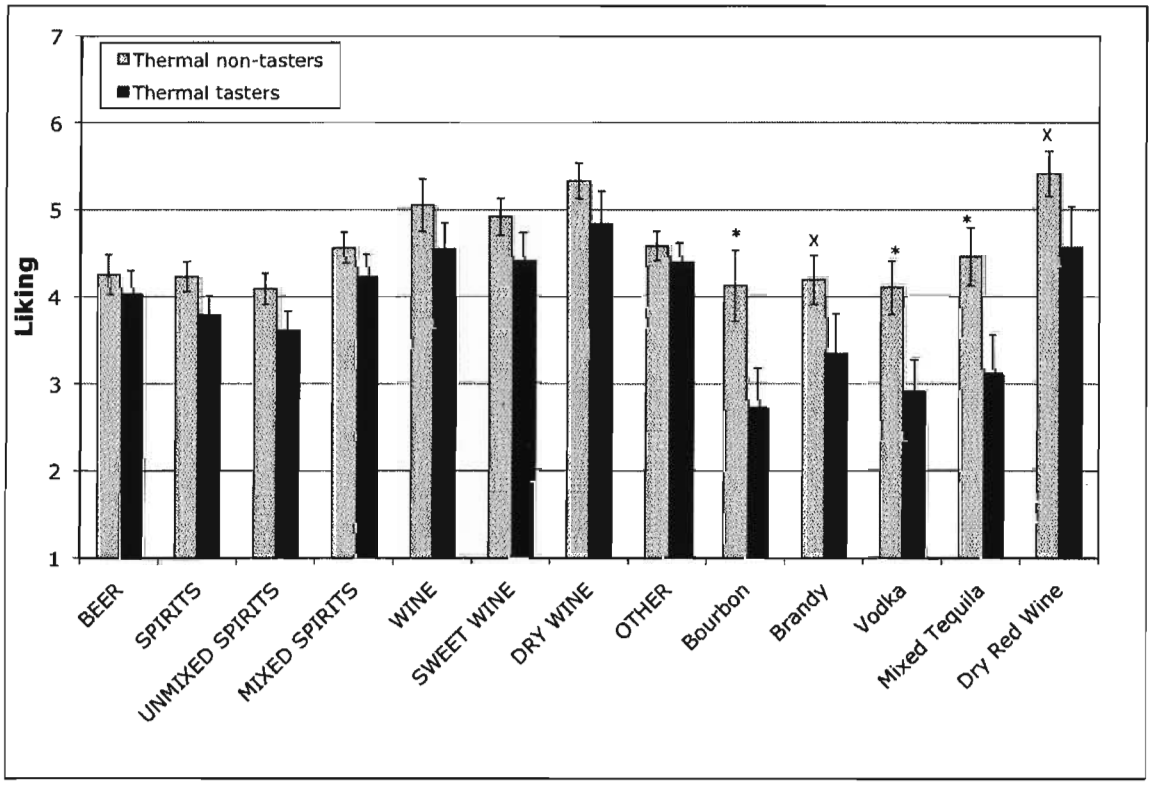
PROP Taster Status (PTS)

Two-way ANOVA revealed that *wine* ($F = 3.24$, 2/122 df, $p < 0.05$) and *sweet wine* ($F = 3.40$, 2/122 df, $p < 0.05$) liking was rated significantly higher by pMTs than pNTs. Liking of sweet red wine was rated significantly higher by pSTs than pNTs, and by pMTs than pNTs ($F = 3.45$, 2/117 df, $p < 0.05$), while sweet white wine received a significantly higher liking rating from pMTs than either pNTs or pSTs ($F = 4.78$, 2/117 df, $p < 0.01$). Liking of mixed tequila ($F = 2.47$, 2/105 df, $p = 0.09$), and fruit wine ($F = 2.56$, 2/105 df, $p = 0.08$) approached significance, with pMTs providing higher ratings than either of the other two groups. Significant PTS*gender interactions were found for sweet red wine ($F = 5.82$, 2/117 df, $p < 0.01$), and tequila ($F = 3.74$, 2/113 df, $p < 0.05$). While female pSTs provided lower liking scores than male pSTs for sweet red wine, the opposite was found for tequila. PTS*gender interactions for ale ($F = 2.66$, 2/106 df, $p = 0.075$), mild/brown ale ($F = 2.54$, 2/90 df, $p = 0.085$) approached significance, with male pSTs rating their liking of these beverages higher than female pSTs.

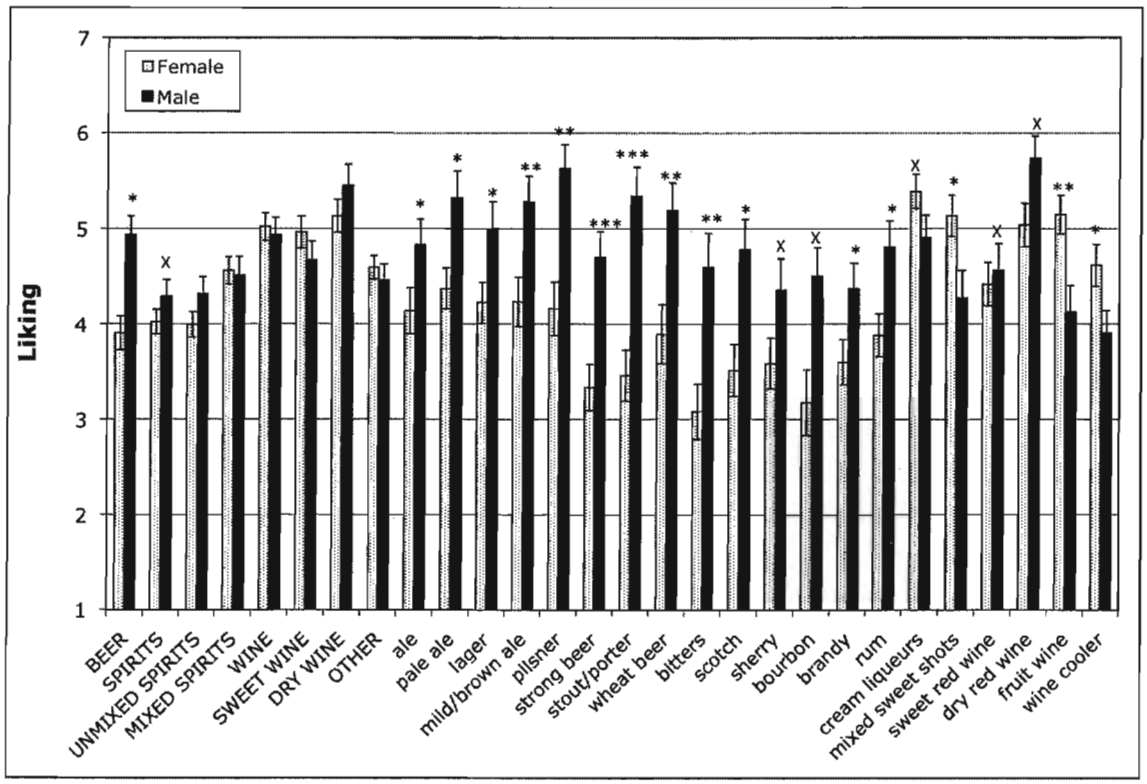
To increase statistical power, variables that were not affected by gender or PTS*gender were subjected to one-way ANOVA examining PTS effects independently. *Overall alcoholic beverage* liking differed significantly between the PTS groups ($F = 3.17$, 2/120 df, $p < 0.05$), with pMTs (mean [SE] = 4.64 [0.16]) providing higher liking scores than pSTs (mean [SE] = 4.14 [0.18]). Dessert/ice wines ($F = 3.18$, 2/112 df, $p < 0.05$) were rated significantly higher by pMTs than pNTs. pMTs' higher liking of rosé/blush wines ($F = 2.63$, 2/113 df, $p = 0.08$) over pNTs approached significance.


PROP non-tasters
PROP medium-tasters
PROP non-tasters
Liking
7
6
5
4
3
2
1
BEER
SPIRITS
UNMIXED SPIRITS
MIXED SPIRITS
OTHER
DRY WINE
WINE
SWEET WINE
sweet red wine
sweet white wine
mixed tequila
fruit wine
dessert/ice wine
rose/blush wine

A)


Thermal non-tasters
Thermal tasters
Liking
7
6
5
4
3
2
1
BEER
SPIRITS
UNMIXED SPIRITS
MIXED SPIRITS
WINE
SWEET WINE
DRY WINE
OTHER
Bourbon
Brandy
Vodka
Mixed Tequila
Dry Red Wine

B)



C)

Fig. 1. A) PROP tasters status, B) thermal taster status, and C) gender effects on liking (measured on a 7-point scale) of alcoholic beverage types (upper-case), and individual alcoholic beverages (lower-case). Bars represent mean intensity ratings ± SE mean. In A), means with different letters differ at the $p<0.05$ level of significance. In B) and C), means that differ significantly at $p<0.1$, $p<0.05$, $p<0.01$, and $p<0.001$ are indicated by X, *, **, or *** respectively.

Thermal Taster Status (TTS)

Two-way ANOVA revealed that TTs rated liking of bourbon ($F = 5.88$, 1/37 df, $p < 0.05$) significantly lower than TnTs. TnTs greater liking of brandy ($F = 3.37$, 1/55 df, $p = 0.07$) and vodka ($F = 3.03$, 1,70 df, $p = 0.09$) approached significance. *Overall alcoholic beverage* liking demonstrated a significant TTS*gender interaction ($F = 6.19$, 1/70 df, $p < 0.05$), with female TnTs providing higher scores than female TTs, and male TnTs providing lower scores than male TTs. A significant TTS*gender interaction was found for overall beer liking ($F = 5.99$, 1/69 df, $p < 0.05$), pilsner beer ($F = 7.03$, 1/45 df, $p < 0.05$), and strong beer ($F = 10.64$, 1/54 df, $p < 0.01$), with male TTs rating it higher than female TTs. The same interaction approached significance for lager beer ($F = 3.86$, 1/66 df, $p = 0.06$), mild/brown ale beer ($F = 3.22$, 1/53 df, $p = 0.08$), stout/porter beer ($F = 4.04$, 1/58 df, $p = 0.05$), and bitters ($F = 3.92$, 1/36 df, $p = 0.06$). TTS*gender approached significance for liking of light beer ($F = 2.92$, 1/63 df, $p = 0.09$) and rum cooler ($F = 3.36$, 1/62 df, $p = 0.07$) liking, with female TnTs providing higher ratings. Wine coolers ($F = 5.92$, 1/66 df, $p < 0.05$) yielded a significant TTS*gender interaction with female TnTs providing higher liking scores than female TTs, and vice versa for males. TTS*gender approached significance for mixed tequila ($F = 3.62$, 1/57 df, $p = 0.06$), with TnT females providing higher liking ratings than TT females.

To increase statistical power, variables that were not affected by gender or TTS*gender were subjected to one-way ANOVA examining TTS effects independently. TnTs rated the liking of mixed tequila ($F = 5.50$, 1/57 df, $p < 0.05$) higher than TTs. TnTs greater liking of dry red wine ($F = 2.93$, 1/70 df, $p = 0.09$) approached significance.

Gender

Two-way ANOVA with PTS and gender as factors revealed that *beer* liking was rated significantly higher by males than females ($F = 14.92$, 1/120 df, $p < 0.001$). Liking of ale ($F = 6.16$, 1/106 df, $p < 0.05$), pale ale ($F = 6.64$, 1/101 df, $p < 0.05$), lager ($F = 6.34$, 1/111 df, $p < 0.05$), mild/brown beer ($F = 8.13$, 1/90 df, $p < 0.01$), pilsner beer ($F = 14.42$, 1/81 df, $p < 0.001$), strong beer ($F = 15.27$, 1/96 df, $p < 0.001$), wheat beer ($F = 7.78$, 1/78 df, $p < 0.01$), stout/porter beer ($F = 19.05$, 1/100 df, $p < 0.001$), bitters ($F =$

11.10, 1/63 df, $p < 0.01$), and scotch ($F = 4.83$, 1/100 df, $p < 0.05$) was rated significantly higher by males than females. Females rated liking of fruit wine ($F = 7.15$, 1/116 df, $p < 0.01$) higher than males. Males higher liking scores for sherry ($F = 2.96$, 1/88 df, $p = 0.09$), bourbon ($F = 3.99$, 1/67 df, $p = 0.05$), dry red wine ($F = 2.84$, 1/120 df, $p = 0.09$), and sweet red wine ($F = 2.92$, 1/117 df, $p = 0.09$) approached significance, as did females higher scores for liking of cream liqueurs ($F = 3.02$, 1/118 df, $p = 0.08$).

To increase statistical power, variables that were not affected by PTS or PTS*gender were subjected to one-way ANOVA examining gender effects independently. Males' greater liking of *spirits* ($F = 3.82$, 1,122 df, $p = 0.05$) approached significance, and males rated liking of rum ($F = 6.39$, 1/117 df, $p < 0.05$) and brandy ($F = 4.20$, 1/116 df, $p < 0.05$) significantly higher than females. Mixed sweet shots ($F = 5.77$, 1/102 df, $p < 0.05$), and wine coolers ($F = 4.33$, 1/102 df, $p < 0.05$) were rated higher by females than males.

Oral Sensations

The perceived intensity of oral sensations, including temperature, were treated as continuous variables allowing for examination of their correlation with liking of alcoholic beverage types and individual alcoholic beverage for each PTS and TTS group separately. For pSTs, (log)sweet intensity was positively associated with liking of *wine* ($r = .39$, $p < 0.05$), *dry wine* ($r = .42$, $p < 0.05$), dessert/ice wine ($r = .46$, $p < 0.05$), sparkling dry wine ($r = .42$, $p < 0.05$), dry red wine ($r = .40$, $p < 0.05$). (log)high metallic was correlated with liking of bitter/sour/spicy mixed shots ($r = -.46$, $p < 0.05$) and dry red wine ($r = .41$, $p < 0.05$). Perceived intensity of bitterness ($r = -.607$, $p < 0.01$), low metallic ($r = -.47$, $p < 0.5$), and (log)high astringency ($r = -.44$, $p < 0.05$) were all negatively correlated with liking of sweet shots. For pMTs, liking of bourbon was positively correlated with (log)sweet intensity ($r = .42$, $p < 0.05$). (log)salt intensity was positively correlated with brandy ($r = .39$, $p < 0.01$) and mixed rum ($r = .31$, $p < 0.05$) liking. (log)sour intensity was negatively correlated with liking of cream liqueurs ($r = -.31$, $p < 0.05$). (log)high metallic associated with mixed rum ($r = .30$, $p < 0.05$). (log)bitter intensity was negatively correlated with sweet shots ($r = -.28$, $p < 0.04$). Liking of mixed vodka was correlated with (log)high astringency ($r = -.30$, $p < 0.05$), and liking of wine coolers was correlated with perceived intensity of (sqrt)warmth ($r = -$

.28, $p < 0.05$). For pNTs, perceived intensity of sour was correlated with stout/porter ($r = -.42$, $p < 0.05$), wheat beer ($r = -.42$, $p < 0.05$), and fruit wine ($r = .44$, $p < 0.05$). Salt intensity was correlated with liking of dry red wine ($r = -.37$, $p < 0.05$), dessert/ice wine ($r = .37$, $p < 0.05$), sweet mixed shots ($r = 0.48$, $p < 0.01$), and bitter/sour/spicy mixed shots ($r = 0.43$, $p < 0.05$). Sweet intensity was negatively correlated with dry red wine liking ($r = -.36$, $p < 0.05$). (log)high metallic intensity was negatively correlated with liking of wheat beer ($r = -.43$, $p < 0.05$). (sqrt)low metallic intensity was correlated with liking of stout/porter ($r = -.45$, $p < 0.05$), wheat beer ($r = -.43$, $p < 0.05$), bitters ($r = -.48$, $p<0.05$), bourbon ($r = -.47$, $p < 0.05$), scotch ($r = .51$, $p < 0.01$), and dry white wine ($r = .38$ $p < 0.05$). (sqrt)high astringency was correlated with liking of *other* beverages ($r = .34$, $p < 0.05$), bitter/sour/spicy mixed shots ($r = .38$, $p < 0.05$) and sweet mixed shots ($r = .39$, $p < 0.05$). (sqrt)low astringency was correlated with liking of bitter/sour/spicy mixed shots ($r = .42$, $p < 0.05$), sweet mixed shots ($r = .37$, $p < 0.05$), and dry red wine ($r = -.38$, $p < 0.05$). Perceived intensity of coolness was correlated with liking of *other* beverages ($r = .36$, $p < 0.05$), wheat beer ($r = -.41$, $p < 0.05$), clear liqueurs ($r = .37$, $p < 0.05$), and sweet mixed shots ($r = .37$, $p < 0.05$). Perceived intensity of warmth was correlated with liking of *other* beverages ($r = .36$, $p < 0.05$), wheat beer ($r = -.45$, $p < 0.05$), and dessert/ice wine ($r = .49$, $p < 0.01$).

Results for TTs and TnTs are presented in Table 1A) and 1B), respectively.

Predicting Alcoholic Beverage Liking

PROP intensity, FP density, age, and gender were included as independent variables in multiple regressions on liking of the *beer, spirits, unmixed spirits, mixed spirits, wine, sweet wine*, and *dry wine* categories (Table 2A). Gender was a significant contributor to the liking of *beer*, while age contributed significantly to liking of *mixed spirits* and *dry wine*. PROP intensity and FP density were not significant contributors to models of liking for any beverage type.

In a separate analysis, TTS, FP density, age, and gender were included as independent variables in multiple regressions on liking of *beer, spirits, unmixed spirits, mixed spirits, wine, sweet wine*, and *dry wine* (Table 2B). Age was a significant

Table 1. Correlations between perceived intensities of taste and non-taste oral stimuli and liking of individual alcoholic beverages for A) thermal non-tasters (n=23-46), and B) thermal tasters (n=16-23). Pearson's r is indicated. All values are significant at $p<0.05$, $p<0.01$ is indicated by italics, and $p<0.001$ is bolded.

A)

Orosensory stimuli	Beverage	R
(sqrt)low astringency	Sherry	.36
(log)high astringency	Pale ale beer	*.41*
	Lager beer	*.42*
	Mild/brown beer	.35
	Strong beer	.36
	Wheat beer	.39
	Cider	.36
	Mixed rum	*.38*
	Rye	.34
	Mixed rye	.31
(sqrt)high metallic	Lager beer	.37
	Mixed rum	.33
Low metallic	Lager beer	.33
	Tequila	.37
	Mixed tequila	.32
(log)bitter	Ale	.33
	Lager beer	*.39*
(log)sweet	Tequila	.32
	Mixed tequila	.33
(log)sour	Rum	.33
	Mixed Rum	.34
(log)PROP	Mixed bourbon	.44
	Sherry	.44
	Dessert/ice wine	.37

	Sparkling dry wine	.39
	Sweet red wine	.32
(sqrt)coolness	Mixed rum	.30
(sqrt)warmth	Cider	.35
	Mixed rum	.30
	Mixed tequila	.34

B)

Orosensory stimuli	Beverage	R
Bitter	Scotch	*.62*
Sweet	*Wine*	**.67**
	Wine sweet	*.61*
	Wine dry	*.53*
	Cider	.56
	Clear liqueurs	.47
	Mixed sweet shots	.50
	Dessert/ice wine	.51
	Dry sparkling wine	.44
	Fruit wine	.45
	Dry red wine	*.53*
	Rose/blush wine	*.58*
	Sweet sparkling wine	.51
(log)sour	Tequila	-.51
	Mixed tequila	*-.58*
(log)PROP	Wheat beer	.50
	Wine cooler	*-.65*

Table 2. FP density (FP/cm^2), age, gender (females = 0, males = 1), and I) PROP intensity or II) thermal taster status (TTS; TTs = 0, TnTs = 1) as predictors of liking of alcoholic beverage types (*beer, spirits, unmixed spirits, mixed spirits, wine, sweet wine,* and *dry wine*). The intercept (A), multiple correlation coefficient (R), independent variable coefficient (B), standardized independent variable coefficient (β), and semipartial correlation (sr) for each dependent variable with a significant parameter are shown. Bolded values indicate significance at the 0.05 level, bolded and italicized indicate 0.01 significance, and bolded, italicized and underlined numbers indicate a 0.001 level of significance

I)

			(sqrt) PROP			Age			Gender			FP/cm^2		
	R	A	B	β	sr	B	β	Sr	B	β	sr	B	β	sr
Beer	***0.40***	3.50	-0.01	-0.03	-0.02	0.01	0.01	0.06	1.24	0.40	***0.40***	0.00	0.03	0.02
Mixed Spirits	**0.30**	5.97	0.02	0.05	0.04	-0.03	-0.30	***-0.29***	-0.09	-0.03	-0.03	-0.01	-0.10	-0.09
Dry Wine	***0.44***	2.84	-0.01	-0.03	-0.02	0.06	0.43	***0.42***	0.36	0.11	0.11	0.01	0.10	0.09

II)

			TTS			Age			Gender			FP/cm^2		
	R	A	B	B	sr	B	β	Sr	B	β	sr	B	β	sr
Spirits	0.39	5.09	0.13	0.21	0.21	-0.03	-0.30	**-0.30**	0.04	0.02	0.02	-0.01	-0.14	-0.14
Mixed Spirits	***0.48***	6.27	0.09	0.14	0.14	-0.04	-0.42	***-0.42***	-0.30	-0.11	-0.11	-0.01	-0.16	-0.16
Dry Wine	**0.45**	3.23	0.15	0.18	0.18	0.05	0.40	***0.40***	0.23	0.06	0.06	-0.01	-0.06	-0.06

contributor to the predicted liking of *dry wine* and *mixed spirits*. TTS, gender, and FP density were not significant contributors to models of liking for any beverage.

Alcoholic Beverage Consumption

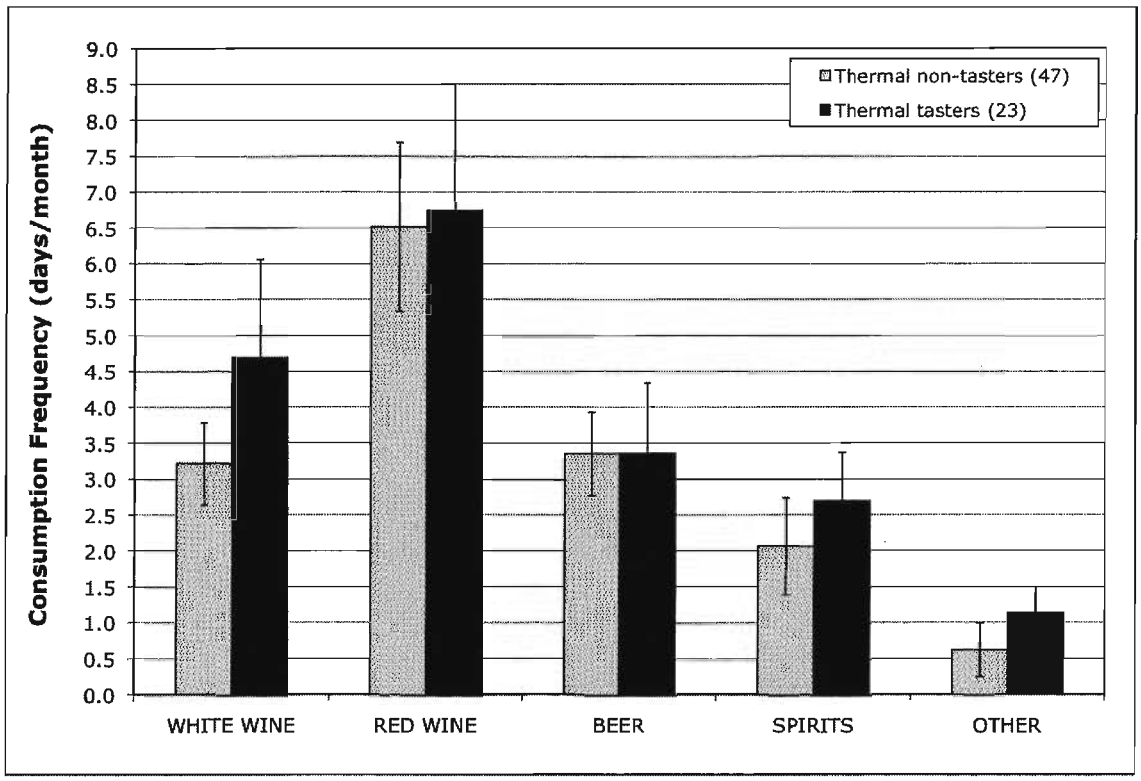
The total number of alcoholic drinks consumed per month (d/m), consumption frequency (F), and quantity of drinks consumed per sitting (Q) for the 5 categories of alcohol were examined. Means and Tukey's HSD results for significant ($p < 0.05$) and near significant ($p < 0.1$) TTS effects on consumption frequency (F), number of drinks per drinking occasion (Q), and number of drinks per month (d/m) are summarized in Figures 2A), B), and C), respectively. For gender these parameters are presented in Figures 3A), B), and C), respectively.

PROP Taster Status (PTS)

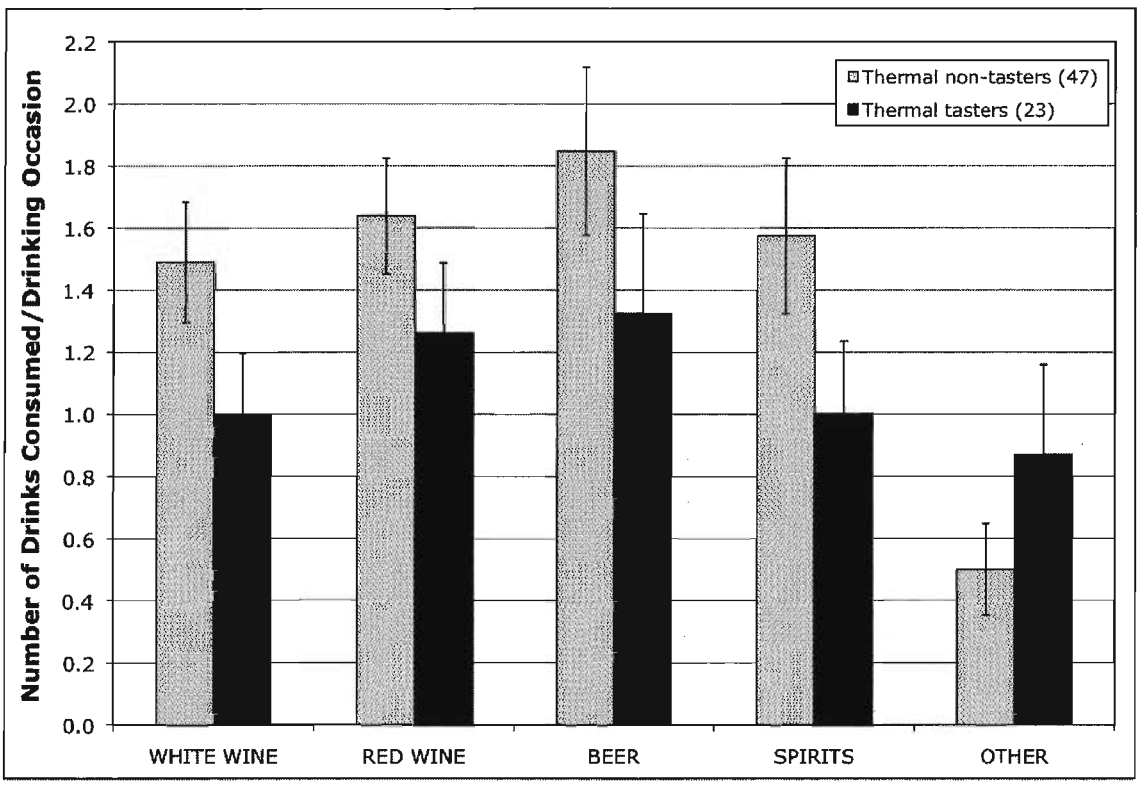
Two-way ANOVA with PTS and gender as factors revealed that pMTs (mean [SE] = 3.3 [0.51]) BEER F was significantly lower than pNTs (mean [SE] = 5.1 [1.1]) and pSTs (mean [SE] = 5.0 [1.5]; $F = 3.6$, 2/119 df, $p < 0.05$), with no significant difference between pNTs and pSTs. Significant PTS*gender interactions were observed for WHITE WINE F ($F = 3.27$, 2/121 df, $p < 0.05$), with pST females having a higher WHITE WINE F than pST males. A PTS*gender interaction for BEER F ($F = 2.59$, 2/119 df, $p = 0.08$) approached significance with pMT males having a higher BEER F than either pST or pNT males. PTS*gender interactions approached significance for OTHER F ($F = 2.4$, 2/119 df, $p = 0.09$) and OTHER d/m ($F = 2.49$, 1/228 df, $p = 0.09$) with pST females consuming more drinks with greater frequency than pST males. To increase statistical power, variables that were not affected by gender or PTS*gender were subjected to one-way ANOVA examining PTS effects independently, which did not reveal any additional PTS effects on alcohol consumption.

Thermal Taste (TTS)

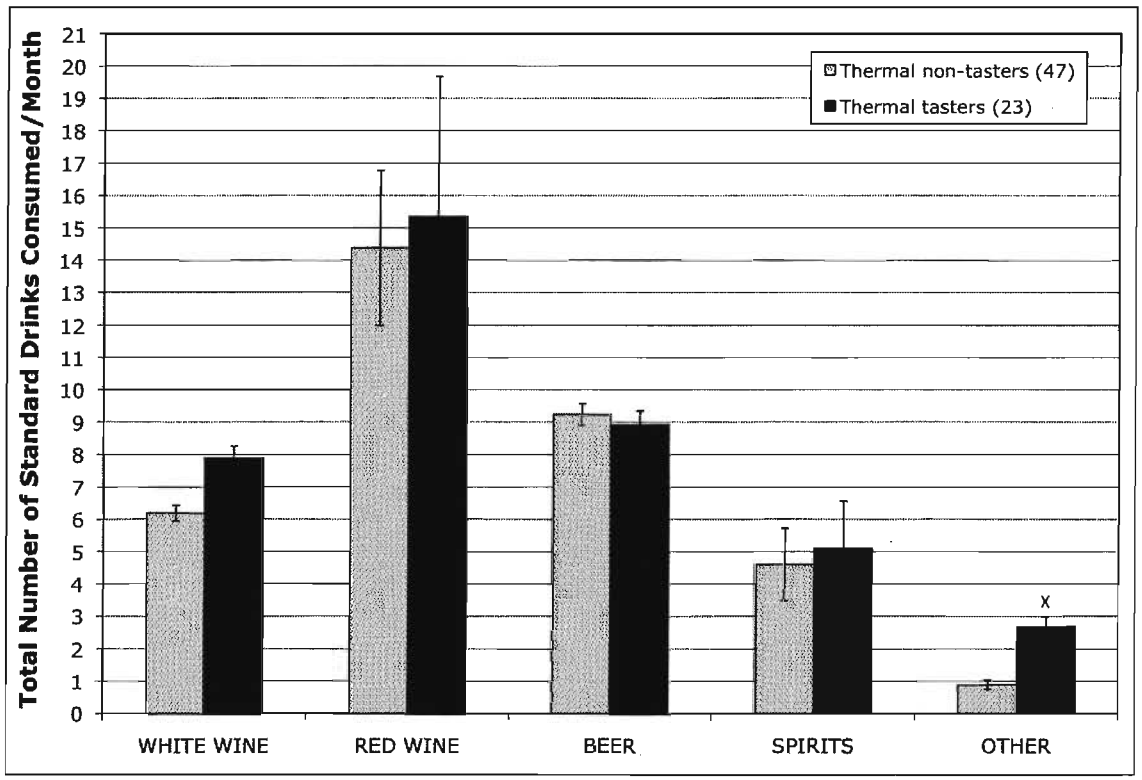
Two-way ANOVA with TTS and gender as factors did not reveal any significant TTS effects on alcoholic beverage consumption behaviour. A TTS*gender interaction was found for BEER F ($F = 4.17$, 1/68 df, $p < 0.05$) and BEER d/m ($F = 4.36$, 1/68 df, $p < 0.05$) with male TTs having a higher BEER F and BEER d/m than female TTs.


Consumption Frequency (days/month)
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Thermal non-tasters (47)
Thermal tasters (23)
WHITE WINE
RED WINE
BEER
SPIRITS
OTHER

A)

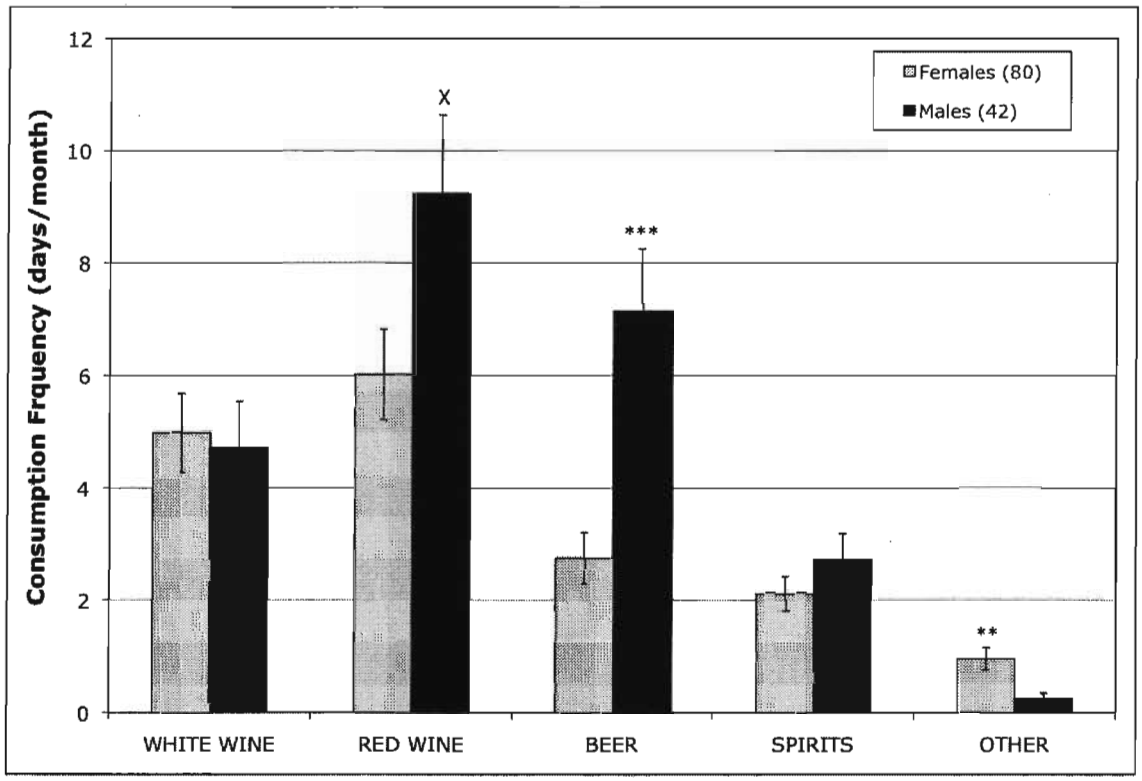

Number of Drinks Consumed/Drinking Occasion
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Thermal non-tasters (47)
Thermal tasters (23)
WHITE WINE
RED WINE
BEER
SPIRITS
OTHER

B)

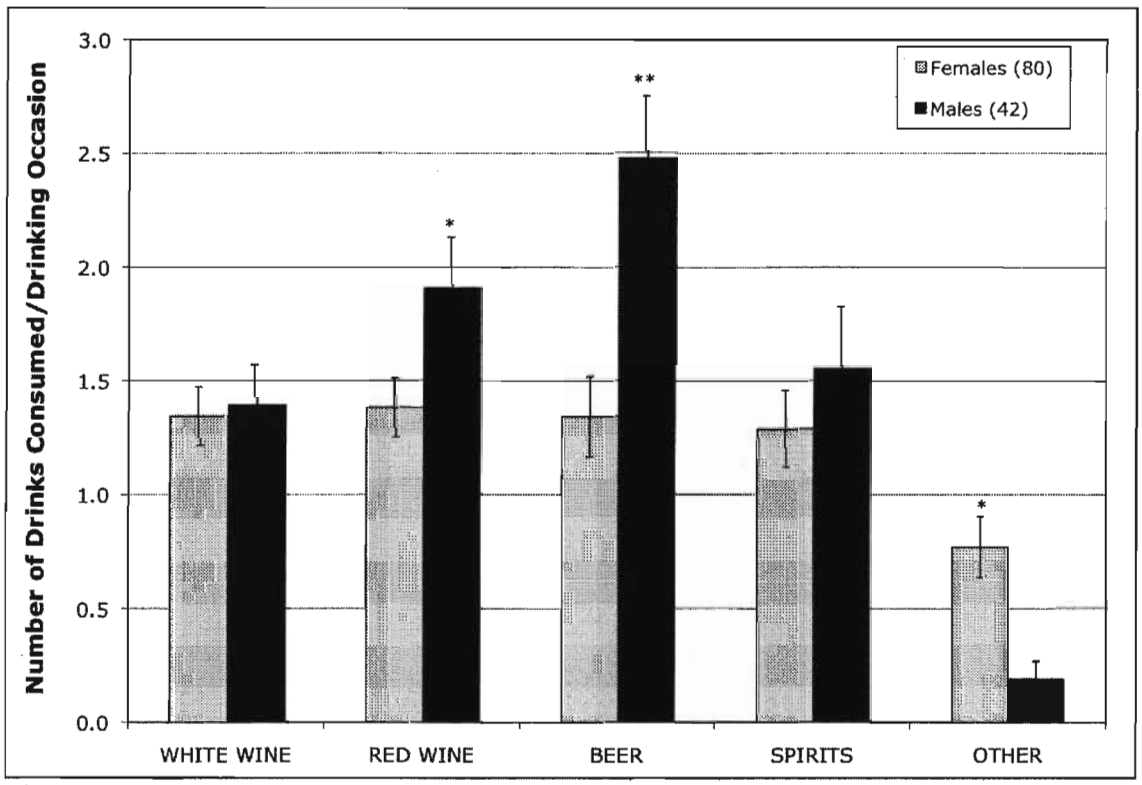


C)

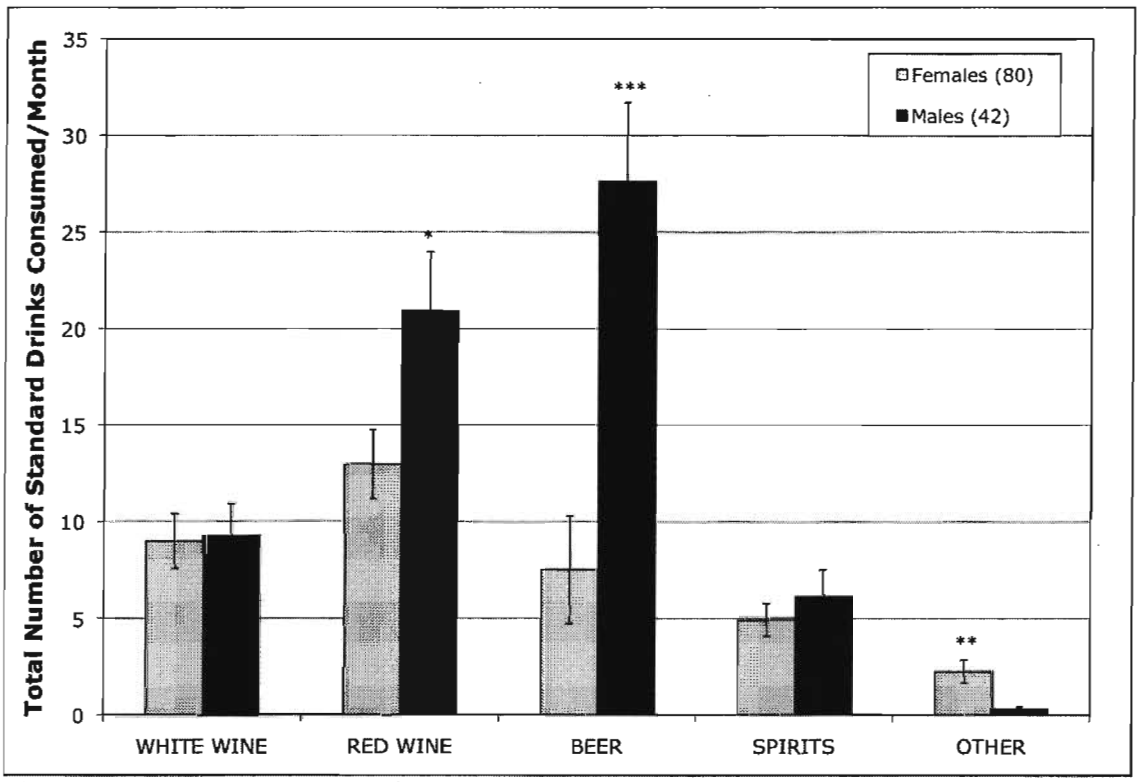
Fig. 2. Thermal taster status effects on A) consumption frequency (days per month), B) number of drinks consumed per drinking occasion, and C) total number of standard drinks consumed per month. Bars represent mean intensity ratings ± SE mean. Differences with a significance of $p<0.1$ are indicated by X.


Consumption Frquency (days/month)
12
10
8
6
4
2
0
X
***
**
Females (80)
Males (42)
WHITE WINE
RED WINE
BEER
SPIRITS
OTHER

A)


Number of Drinks Consumed/Drinking Occasion
3.0
2.5
2.0
1.5
1.0
0.5
0.0
**
*
*
Females (80)
Males (42)
WHITE WINE
RED WINE
BEER
SPIRITS
OTHER

B)



C)

Fig. 3. Gender effects on A) consumption frequency (F) in days per month, B) number of drinks consumed per drinking occasion (Q), and C) total number of standard drinks consumed per month (d/m). Bars represent mean intensity ratings ± SE mean. Differences with a significance of $p<0.1$, $p<0.05$, $p<0.01$, $p<0.001$ are indicated by x, *, **, ***, respectively.

A TTS* gender interaction was also found for SPIRITS F ($F = 4.94$, 1/68 df, $p < 0.05$), with male TTs having a higher SPIRITS F than male TnTs. To increase statistical power, variables that were not affected by gender or TTS*gender were subjected to one-way ANOVA examining TTS effects independently, which revealed that TTs higher OTHER d/m ($F = 2.82$, 1/68 df, $p = 0.09$) approached significance.

Gender

Two-way ANOVA with PTS and gender as factors revealed that males' BEER F ($F = 23.55$, 1/119 df, $p < 0.001$) was higher than females'. The same relationship approached significance for RED WINE F ($F = 3.52$, 1/121 df, $p = 0.06$). Males also had a higher BEER Q ($F = 14.06$, 2/119 df, $p < 0.001$) than females. This relationship also approached significance for RED WINE Q ($F = 3.20$, 1/121 df, $p = 0.08$). Females reported a higher OTHER Q ($F = 8.51$, 1/121 df, $p < 0.01$), and a higher OTHER F ($F = 8.87$, 1/119 df, $p < 0.01$) than males. Females consumed more OTHER d/m ($F = 7.76$, 1/118 df, $p < 0.01$) than males, while males consumed more RED WINE d/m ($F = 4.91$, 1/121 df, $p < 0.05$), BEER d/m ($F = 16.55$, 1/121 df, $p < 0.001$). Males (mean [SE] = 60.3 [6.9]) also consumed more TOTAL d/m than ($F = 11.56$, 1/120 df, $p < 0.01$) than females (mean [SE] = 33.4 [3.2]). To increase statistical power, variables that were not affected by PTS or PTS*gender were subjected to one-way ANOVA examining gender effects independently, which did not reveal any additional gender effects on alcohol consumption.

Oral Sensations

The perceived intensity of oral sensations, including temperature, were treated as continuous variables allowing for examination of their correlation with parameters of alcoholic beverage consumption for each PTS and TTS group separately. For pNT, BEER F was correlated with the perceived intensity of sour ($r = -.36$, $p < 0.05$) and (log)high metallic ($r = -.36$, $p < 0.05$). RED WINE Q was correlated with (sqrt)low astringency ($r = -.35$, $p < 0.05$). For pMTs, (log)low metallic was correlated with (sqrt)SPIRITS F ($r = .31$, $p < 0.05$) and (sqrt)SPIRITS d/m ($r = .27$, $p < 0.05$). For pSTs, RED WINE F was correlated with the perceived intensities of (log)high astringency ($r = .39$, $p < 0.05$), (log)high metallic ($r = .45$, $p < 0.05$), (log)sweet ($r = .52$, $p < 0.05$),

(log)coolness ($r = .41$, $p < 0.05$), and (log)warmth ($r = .47$, $p < 0.05$). RED WINE Q was correlated with the intensities of low metallic ($r = .39$ $p < 0.05$), (log)high metallic ($r = .43$, $p < 0.05$), (log)sweet ($r = .44$, $p < 0.05$), (log)coolness ($r = .43$, $p < 0.05$), and (log)warmth ($r = .39$, $p < 0.05$). RED WINE d/m was correlated with the intensities of (log)high astringency ($r = .42$, $p < 0.05$), low metallic ($r = .46$ $p < 0.05$), (log)high metallic ($r = .50$, $p < 0.01$), (log)sweet ($r = .51$, $p < 0.01$), (log)coolness ($r = .56$, $p < 0.01$), and (log)warmth ($r = .57$, $p < 0.01$).

Results for TTs and TnTs are presented in Table 3A) and B), respectively.

Predicting Alcoholic Beverage Consumption

PROP intensity, FP density, age, and gender were included as independent variables in multiple regressions on consumption frequency, number of drinks consumed per sitting, and number of drinks consumed per month for each type of alcoholic beverage (i.e., RED WINE, WHITE WINE, SPIRITS, BEER, OTHER; Table 4A). Gender, age, and FP density were significant contributors to RED WINE F, while gender and age were significant contributors to RED WINE d/m, and OTHER d/m. Gender alone was a significant contributor to BEER F, Q and d/m, as well as RED WINE Q, and OTHER Q and d/m. Age alone was a significant contributor to WHITE WINE F and WHITE WINE d/m. PROP intensity was not a significant contributor to any consumption parameters examined.

TTS, FP density, age, and gender were included as independent variables in multiple regressions on consumption frequency, number of drinks consumed per sitting, and number of drinks consumed per month for each type of alcoholic beverage (i.e., RED WINE, WHITE WINE, SPIRITS, BEER, OTHER; Table 4B). TTS and age were significant contributors to OTHER F and d/m. Gender was a significant contributor to BEER F, Q and d/m. Age was a significant contributor to WHITE WINE F, RED WINE d/m, and TOTAL d/m.

Liking and Consumption

The relationship between liking and consumption of each alcohol type was examined using Pearson's r. *Beer* liking was significantly correlated with (sqrt)BEER F ($r = .58$, $p < 0.0001$), BEER Q ($r = .46$, $p < 0.0001$), BEER d/m ($r = .36$, $p < 0.0001$),

Table 3. Correlations between perceived intensities of taste and non-taste oral stimuli and consumption parameters (consumption frequency (F), number of drinks per drinking occasion (Q), and number of drinks per month (d/m)) of individual alcoholic beverages for A) thermal non-tasters (n=43-46), and B) thermal tasters (n=21-23). Pearson's r is indicated. All values are significant at $p<0.05$, $p<0.01$ is indicated in italics, and $p<0.001$is indicated in bold.

A)

Orosensory stimuli	Consumption parameter	r
(log)bitter	BEER Q	.34
(log)PROP	(sqrt)WHITE WINE F	.30

B)

Orosensory stimuli	Consumption parameter	r
High astringency	RED WINE d/m	.43
Bitter	RED WINE F	.42
	RED WINE Q	*.56*
	RED WINE d/m	.42
	SPIRITS F	.43
	TOTAL d/m	.44
Sweet	RED WINE F	.47
	RED WINE Q	*.53*
	RED WINE d/m	.48
	OTHER F	.49
	OTHER d/m	-.50
(log)sour	RED WINE F	*.44*
	RED WINE Q	.44
	RED WINE d/m	.46
(log)PROP	RED WINE Q	.43
(log)cooling	RED WINE d/m	.42
(log)warming	RED WINE Q	.42
	RED WINE d/m	.43

Table 4. FP density (FP/cm^2), age, gender (females = 0, males = 1), and I) PROP intensity or II) thermal taster status (TTS; TTs = 0, TnTs = 1) as predictors of alcoholic beverage category (WHITE WINE, RED WINE, BEER, SPIRITS, OTHER, TOTAL) consumption parameters (consumption frequency (F), number of drinks per drinking occasion (Q), and number of standardized drinks per month (d/m)). The intercept (A), multiple correlation coefficient (R), independent variable coefficient (B), standardized independent variable coefficient (β), and semipartial correlation (sr) for each dependent variable with a significant parameter are shown. Bolded values indicate significance at the 0.05 level, bolded and italicized indicate 0.01 significance, and bolded, italicized and underlined numbers indicate a 0.001 level of significance.

A)

			(sqrt)PROP			Age			Gender			FP/cm^2		
	R	A	B	β	sr	B	β	Sr	B	β	sr	B	β	sr
WHITE WINE-d/m	***0.36***	-4.85	0.37	0.10	0.09	0.32	0.32	***0.31***	1.14	0.05	0.05	0.02	0.04	0.03
RED WINE-F	***0.54***	-10.39	0.23	0.09	0.08	0.28	0.41	***0.40***	4.18	0.24	***0.24***	0.08	0.20	**0.18**
RED WINE-d/m	***0.51***	-19.31	0.57	0.11	0.09	0.56	0.37	***0.36***	9.68	0.26	***0.26***	0.14	0.16	0.14
BEER-Q	***0.46***	1.27	-0.03	-0.07	-0.06	-0.02	-0.17	-0.17	1.41	0.40	***0.40***	0.01	0.14	0.13
BEER-d/m	***0.44***	2.45	-0.73	-0.09	-0.08	-0.11	-0.05	-0.05	23.49	0.43	***0.43***	0.16	0.12	0.10
TOTAL-d/m	***0.46***	-0.06	-0.43	-0.04	-0.03	0.35	0.11	0.11	34.45	0.43	0.43	0.32	0.17	0.15
(sqrt)WHITE WINE-F	***0.16***	-0.38	0.03	0.07	0.06	0.04	0.35	***0.34***	0.06	0.02	0.02	0.01	0.12	0.11
(sqrt)RED WINE-Q	***0.38***	0.45	0.04	0.19	0.17	0.01	0.19	0.19	0.35	0.25	***0.25***	0	-0.02	-0.02
(sqrt) BEER-F	***0.47***	0.21	-0.03	-0.06	-0.06	0.00	0.04	0.04	1.33	0.46	***0.46***	0.01	0.18	0.16
(sqrt) SPIRITS-Q	0.25	1.43	0.02	0.08	0.07	-0.01	-0.22	-0.21	0.17	0.11	0.11	0	-0.09	-0.08
(sqrt) OTHER-F	**0.33**	0.93	0.02	0.09	0.08	-0.01	-0.22	-0.21	-0.39	-0.25	**-0.25**	0	-0.19	-0.02
(sqrt) OTHER-d/m	**0.33**	1.48	0.04	0.13	0.11	-0.02	-0.22	**-0.22**	-0.06	-0.26	***-0.25***	0	-0.07	-0.07
(sqrt) OTHER-Q	**0.35**	0.96	0.02	0.08	0.07	-0.01	-0.22	-0.22	-0.37	-0.28	***-0.28***	0	-0.07	-0.06

B)

			TTS			Age			Gender			FP/cm^2		
	R	A	B	β	sr	B	β	sr	B	B	sr	B	β	sr
(sqrt)WHITE WINE-F	**0.47**	-0.05	-0.08	-0.12	-0.12	0.04	0.40d	***0.40***	0.20	0.071	0.07	0.01	0.12	0.12
RED WINE-F	***0.53***	-8.34	-0.13	-0.03	-0.03	0.33	0.49	0.49	3.52	0.19	0.19	0.06	0.13	0.13
RED WINE-d/m	***0.47***	-9.32	-0.58	-0.06	-0.07	0.65	0.44	***0.44***	6.17	0.155	0.15	0.05	0.05	0.05
(sqrt)BEER-F	0.39	1.03	0.05	0.07	0.07	0.01	0.06	0.061	1.05	0.385	***0.38***	0	-0.07	-0.07
(sqrt)BEER-Q	0.36	0.83	0.07	0.16	0.16	-0.01	-0.16	-0.159	0.53	0.294	**0.29**	0	0.04	0.04
(sqrt)BEER-d/m	**0.41**	1.09	0.19	0.16	0.16	0	0	0.003	2.06	0.399	***0.39***	0	-0.04	-0.04
(sqrt)OTHER-F	**0.44**	1.58	-0.10	-0.25	**-0.25**	-0.02	-0.32	**-0.32**	-0.36	-0.218	-0.22	0	-0.02	-0.02
OTHER-Q	**0.44**	2.23	-0.14	-0.24	-0.24	-0.03	-0.30	-0.30	-0.61	-0.256	-0.25	0	-0.02	-0.02
(sqrt)OTHER-d/m	***0.47***	2.52	-0.16	-0.27	**-0.26**	-0.03	-0.33	***-0.33***	-0.68	-0.259	**-0.26**	0	-0.01	-0.01
TOTAL/d/m	0.36	10.68	0.46	0.03	0.03	0.77	0.27	0.27*	19.19	0.25	0.25	-0.08	-0.04	-0.04

(sqrt)OTHER F ($r = -.36$, $p < 0.0001$), RED WINE F ($r = .21$, $p < 0.05$), RED WINE Q ($r = .35$, $p < 0.0001$), RED WINE d/m ($r = .23$, $p < 0.05$), and tot d/m ($r = .45$, $p < 0.000001$). Liking of *spirits* was significantly correlated with SPIRITS F ($r = .31$, $p < 0.001$), (sqrt)SPIRITS Q (.31, $p < 0.001$), (sqrt)SPIRITS d/m ($r = .31$, $p < 0.001$), and TOTAL d/m ($r = .25$, $p < 0.05$). *Unmixed spirits* liking was significantly correlated with (sqrt)RED WINE Q ($r = .20$, $p < 0.05$), SPIRITS F ($r = .32$, $p < 0.001$), (sqrt)SPIRITS Q (.29, $p < 0.01$), (sqrt)SPIRITS d/m ($r = .29$, $p < 0.01$), (sqrt)OTHER F ($r = .22$, $p < 0.05$), and tot d/m ($r = .25$, $p < 0.01$). Liking of *mixed spirits* was correlated with SPIRITS F ($r = .25$, $p < 0.05$), (sqrt)SPIRTS Q ($r = .29$, $p < 0.01$), and (sqrt)SPIRITS d/m ($r = .29$, $p < 0.01$). *Wine* liking was significantly correlated with (sqrt)WHITE WINE F ($r = .41$, $p < 0.0001$), WHITE WINE Q ($r = .39$, $p < 0.0001$), WHITE WINE d/m ($r = .27$, $p < 0.01$), RED WINE F ($r = .21$, $p < 0.05$), (sqrt)RED WINE Q ($r = .44$, $p < 0.00001$), RED WINE d/m ($r = .24$, $p < 0.05$), (sqrt)OTHER F ($r = -.22$, $p < 0.05$), (sqrt)OTHER Q ($r = -.23$, $p < 0.05$), (sqrt)OTHER d/m ($r = -.24$, $p < 0.05$), and tot d/m ($r = .21$, $p < 0.05$). Liking of *sweet wine* was correlated with (sqrt)WHITE WINE F ($r = .35$, $p < 0.05$), WHITE WINE Q ($r = .31$, $p < 0.001$), and (sqrt)RED WINE Q ($r = .23$, $p < 0.05$). *Dry wine* liking was correlated with (sqrt)WHITE WINE F ($r = .54$, $p < 0.00001$), WHITE WINE Q ($r = .37$, $p < 0.0001$), WHITE WINE d/m ($r = .46$, $p < 0.000001$), RED WINE F ($r = .52$, $p < 0.00001$), (sqrt)RED WINE Q ($r = .63$, $p < 0.0000001$), RED WINE d/m ($r = .52$, $p < 0.0000001$), (sqrt)BEER F ($r = .22$, $p < 0.05$), (sqrt)OTHER F ($r = -.39$, $p < 0.0001$), (sqrt)OTHER Q ($r = -.39$, $p < 0.0000001$), (sqrt)OTHER d/m ($r = -.33$, $p < 0.001$), and TOTAL d/m ($r = .41$, $p < 0.00001$). Liking of *other* was correlated with SPIRITS F ($r = 0.21$, $p < 0.05$), and (sqrt)SPIRITS d/m ($r = .21$, $p < 0.05$). *Overall alcoholic beverage* liking was correlated with WHITE WINE Q ($r = .32$, $p < 0.001$), (sqrt)WHITE WINE F ($r = .24$, $p < 0.01$), WHITE WINE d/m ($r = .21$, $p < 0.05$), (sqrt)RED WINE Q ($r = .34$, $p < 0.001$), BEER Q ($r = .20$, $p < 0.05$), (sqrt)BEER F ($r = .25$, $p < 0.01$), SPIRITS F ($r = .27$, $p < 0.01$), (sqrt)SPRITS d/m ($r = .23$, $p < 0.05$), (sqrt)OTHER F ($r = -.26$, $p < 0.01$), (sqrt)OTHER Q ($r = .21$, $p < 0.05$), (sqrt)OTHER d/m ($r = -.20$, $p < 0.05$), and TOTAL d/m ($r = .32$, $p < 0.001$).

Discussion

Alcoholic Beverage Liking

Although only significant for *wine* and *sweet wine*, a trend of pMTs liking all beverage types more than either pNTs or pSTs was observed. The individual alcoholic beverages that differed significantly between the three PTS groups were liked more by pMTs, and tended to be predominantly sweet in taste. Given the pST protection hypothesis (Duffy et al., 2004a; Intranuovo and Powers, 1998; Driscoll et al., 2006), it was expected that pNTs would like alcoholic beverages more than either of the other two groups. While PROP concentration and PTS categorization may factor into the deviation of this result from expected, psychophysical and physiological data from this cohort would indicate that is not the issue (Bajec and Pickering, 2008). We suggest that pMTs' higher responsiveness to orosensory stimuli compared to pNTs and their lower responsiveness compared to pSTs may confer a benefit in liking of alcoholic beverages. pMTs relative position between the two extreme PTS groups may provide them a 'best of both worlds' advantage, where the perceived intensity of the sensory experience allows them to enjoy the complexity of alcoholic beverages without being overpowered by the bitterness and burn elicited by the ethanol. As pMTs are the largest PTS group, and presumably the largest consumer group, this finding warrants further investigation. It was hypothesized that FP density would be predictive of alcoholic beverage liking (Duffy et al., 2004b), however multiple regression including both PROP and FP density as independent variables failed to predict liking. Additionally, based on the results of Lanier et al. (2005), who found sampled alcoholic beverage bitterness to be a good predictor of preference, it was expected that pSTs' perceived intensity of quinine bitterness may be inversely correlated with alcoholic beverage liking, and that pNTs' perceived intensity of sucrose sweetness might be inversely correlated with alcoholic beverage liking. This was not observed overall, suggesting that the perceived intensity of prototypical tastants may not be an adequate proxy for the intensity of tastes from sampled alcoholic beverages.

Although not significant, a trend for TnTs to like all alcoholic beverage types

more than TTs was observed. Based on the protection hypothesis put forth for pSTs, this finding was expected given TnTs' lower responsiveness to prototypical tastants, flavours, and astringent stimuli (Bajec and Pickering, 2008; Green and George, 2004). The individual beverages that TnTs rated higher than TTs tended to be high alcohol (e.g, bourbon), and astringent (i.e., dry red wine) in nature. Although differences in the perceived intensity of ethanol between the thermal taster groups have not been examined, TTs' higher responsiveness to thermal heat on the tongue (Bajec and Pickering, 2008; Green and George, 2004) may be related to their greater dislike of high alcohol beverages. TnTs perceived temperature intensity associated with the liking of some mixed spirits, which was not observed for TTs, suggesting that temperature may play a role in TTs' decreased liking of some beverages. TTS was not a predictor of alcoholic beverage liking in multiple regression analysis. Interestingly, however, the perceived intensity of prototypical tastants, astringency, metallic flavour and temperature were correlated with liking for a greater number of alcoholic beverages in TnTs, suggesting that orosensory perception may have more influence on TnTs' liking of alcoholic beverages than for TTs.

Based on previous studies examining alcoholic consumption (Barefoot et al., 2002; Klatsky et al., 1983; Tjønneland et al., 1999; Grønbæk et al., 2000), the trends observed here of males liking *beer*, *spirits*, and *dry wine* more than females, and females liking *wines, sweet wine, and mixed spirits* more than males were expected. Liking of individual alcoholic beverages followed this trend and indicates separation of the genders based on the sweetness of the beverage, with males liking beer products and high alcohol spirits more, and females liking creamy and sweet beverages more. Additionally, multiple regression analysis suggests that gender is a good predictor of *beer* liking.

Alcoholic Beverage Consumption

pSTs did not differ from pMTs or pNTs in any parameters of consumption examined, including their intake of total number of standard drinks per month, in contrast to the results of Guinard et al. (1996), Intranuovo and Powers (1998), and Duffy et al. (2004a; 2004b). As noted above, it seems unlikely that PROP concentration or PTS

categorization methodology is responsible for this discrepancy. Additionally, PROP intensity was positively associated with some consumption parameters for both RED and WHITE WINE (data not shown), and in pSTs increased responsiveness to quinine bitterness was not negatively associated with consumption parameter for any of the beverage categories examined. While PROP intensity was not a significant contributor to predicted alcohol consumption parameters, FP density explained an additional 3% of the variance in RED WINE F. A number of studies have failed to report PTS affects on alcoholic beverage intake, or alcoholism (Mattes and DiMeglio, 2001; Kranzler et al., 1996; Kranzler et al., 1998), confirming that alcohol intake parameters have a complex, multifaceted basis, which likely varies greatly between individuals (de Wit et al., 1987).

TTs' higher responsiveness to orosensory stimuli (Bajec and Pickering, 2008), particularly bitterness and thermal heat, led us to hypothesize that TTs' alcoholic beverage intake would be significantly less than TnTs, however, this was not found. TTS contributed significantly to the prediction of OTHER d/m, and interestingly, more consumption parameters for TTs than TnTs were significantly correlated with perceived intensity of orosensory stimuli, perhaps suggesting that taste has more influence on intake for tasters than non-tasters.

While it was hypothesized that males would consume more BEER than females (Barefoot et al., 2002; Klatsky et al., 1983; Tjønneland et al., 1999; Grønbæk et al., 2000), males also consumed more RED WINE than females, which was not expected in a North American population. Females' greater consumption of OTHER, which included drinks such as coolers, ciders, liqueurs, and mixed drinks, suggests that females may be more diverse in their alcoholic beverage choices than males. Gender was a significant contributor to many consumption parameters in both the PTS and TTS regression models, with males' positively associated with all consumption parameters except for OTHER.

Other Considerations

Although not observed for all beverage types, liking and intake of similar alcoholic beverage categories were associated (Pearson's r). Additionally, TOTAL d/m and *overall alcoholic beverage* liking were associated with each other, and with liking or

intake, respectively, of most beverage types examined, suggesting that the use of liking as a proxy for intake may be a useful measure, and should be further examined (Duffy et al., 2009).

Age was a significant predictor of liking and consumption for many types of alcoholic beverages. Interestingly, the attributes of the beverages positively associating with age are predominately bitter and astringent, and those negatively associating with age are predominately sweet. This finding, coupled with the age range of the cohort examined here, suggests that orosensory stimuli initially perceived as aversive become more hedonically acceptable with age (Ton Nu et al., 1996; Rozin and Vollmecke, 1986; Mojet et al., 2005).

For *overall alcoholic beverage* liking and liking of alcoholic beverage categories, Eta-squared (η^2) values are larger for PTS than for TTS and gender (Table 5A), suggesting that PTS has a greater effect on liking than either TTS or gender. Interestingly, for TOTAL d/m, gender had the greatest influence, however, η^2 values for consumption parameters of each beverage category indicate that PTS, TTS, and gender affect consumption differently depending on the alcoholic beverage category (Table 5B). This suggests that consumption of alcoholic beverage types, and consumption overall is more complex than liking, requiring the examination of additional variables to understand the drivers of alcoholic beverage consumption.

Table 5. Eta-squared (η^2) values for PROP taster status (PTS η^2), thermal taster status (TTS η^2) and gender (Gen η^2) effects on A) liking ratings, and B) consumption parameters.

A)

Liking	**TTS η^2**	**PTS η^2**	**Gen η^2**
Beer	6.0E-4	0.038	0.034
Spirits	0.001	0.052	2.81E-7
Unmixed Spirits	0.003	0.047	0.002
Mixed Spirits	1.78E-5	0.048	0.007
Wine	0.007	0.031	0.002
Sweet Wine	0.004	0.023	0.007
Dry Wine	0.010	0.018	0.002
Other	0.007	0.074	0.019
Overall Alcoholic Beverage	1.82E-4	0.073	4.63E-4

B)

Consumption	**TTS η^2**	**PTS η^2**	**Gen η^2**
WHITE WINE-F	0.015	0.003	1.24E-4
WHITE WINE-Q	0.038	0.005	0.014
WHITE WINE-d/m	0.003	0.015	0.002
RED WINE-F	0.001	0.002	0.002
RED WINE-Q	0.01	0.031	0.01
RED WINE-d/m	0.002	0.003	0.008
BEER-F	0.007	0.002	0.038
BEER-Q	0.042	0.062	0.085
BEER-d/m	0.002	0.012	0.018
SPIRITS-F	0.023	0.003	0.006
SPIRITS-Q	0.07	0.08	0.031
SPIRITS-d/m	4.67E-4	0.015	0.001
OTHER-F	0.013	0.023	0.048
OTHER-Q	0.006	0.006	0.052
OTHER-d/m	0.003	0.028	0.036
TOTAL-d/m	9.72E-5	3.66E-3	0.017

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CHAPTER 6: ASSOCIATION OF THERMAL TASTE AND PROP RESPONSIVENESS WITH FOOD LIKING, NEOPHOBIA, BODY MASS INDEX, AND WAIST CIRCUMFERENCE

Introduction

A number of diseases and illnesses, including cardiovascular disease and diabetes, have been either directly or indirectly linked to diet (Kaput, 2004; Low & Tai, 2007; Hu, Manson, Mier, Colditz, Liu, Solomon, & Willett, 2001). Along with demographic, environmental, and cognitive considerations, food preference and liking are important factors guiding food choice and behaviour (Tepper, White, Koelliker, Lanzara, D'Adamo & Gasparini, 2009; Drewnowski, Henderson, Hann, Barratt-Fornell & Ruffin, 1999a; Drewnowski & Hann, 1999b; Ly & Drewnowski, 2001). Recent work strongly suggests that self-reported liking is a valuable measure of food intake as it lacks the reporting and recall biases of self-reported intake (Sullivan, Hayes & Duffy, 2007; Duffy, Hayes, Sullivan & Faghri, 2009). Glanz and colleagues (Glanz, Basil, Maiback, Goldberg & Snyder, 1998) found that 'taste', in this case understood to comprise somatosensory, olfactory, and gustatory sensations, may be the most important predictor of dietary choice. The perception of orosensory (i.e., somatosensory, olfactory, and gustatory) stimuli, which integrate to create flavour (Duffy, 2007; Rozin, 1982), varies greatly between individuals (Schutz & Pilgrim, 1957; Buettner, 2002; Noble, 1995; Leach & Noble, 1986; Manrique & Zald, 2006). Genetic variation is a major contributor to individual differences in the perception of taste stimuli (reviewed in: Garcia-Bailo, Toguri, Eny & El-Sohemy, 2009). The most studied and best-understood genetic source of individual variation in oral sensation is 6-n-propylthiouracil (PROP) responsiveness (reviewed in: Tepper et al. 2009; Duffy, 2007; Tepper, 2008).

PROP responsiveness is typically expressed categorically as PROP taster status (PTS), which consists of three groups: PROP super-tasters (pSTs), PROP medium-tasters (pMTs), and PROP non-tasters (pNTs) (Bartoshuk, 1993), with pSTs being most responsive to the bitterness of PROP, pNTs least responsive, and pMTs presenting intermediate responsiveness. Molecular data indicate that the *TAS2R38* gene encodes

two major forms of the PROP receptor, PAV and AVI; those individuals that carry two PAV alleles are very responsive to PROP, those with two AVI alleles are minimally or non-responsive, and those with one PAV allele and one AVI allele demonstrate intermediate responsiveness (Duffy et al. 2004a). Besides PROP, PTS is also associated with responsiveness to other orosensory stimuli. pSTs perceive prototypical tastants (e.g., salt, sugar, acid), including other bitterants, with greater intensity than pNTs (Tepper et al. 2009; Gent & Bartoshuk, 1983; Bartoshuk, Duffy, Lucchina, Prutkin & Fast, 1998; Prescott, Ripandelli & Wakeling, 2001; Bajec & Pickering, 2008a; Hayes, Bartoshuk, Kidd & Duffy, 2008). Irritation from ethanol (Duffy et al. 2004a; Bartoshuk et al. 1993; Prescott & Swain-Campbell, 2000; Duffy, Peterson & Bartoshuk, 2004b; Bartoshuk, Duffy & Miller, 1994) and the tactile sensation of astringency (Bajec et al. 2008a; Pickering, Haverstock & DiBattista, 2006) are also perceived with greater intensity by those that perceive PROP to be more bitter. Evidence also suggests that pSTs perceive retronasal aroma, and thermal stimuli on the tongue surface more intensely than pNTs and pMTs (Bajec et al. 2008a).

PROP is hypothesized to mediate food choice via taste perception and food preference, and to contribute to disease via diet, and/or through the development of obesity, which is associated with increased disease risk (Tepper, 2004; Duffy et al., 2004c). The increased responsiveness of pSTs to tastant solutions appears to translate into increased responsiveness to those same taste qualities in food. pSTs perceive bitterness (Tepper et al. 2009; Sandell & Breslin, 2006; Dinehart, Hayes, Bartoshuk, Lanier & Duffy, 2006; Akella, Henderson & Drewnowski, 1997; Intranuovo, & Powers, 1998; Lanier, Hayes & Duffy, 2005; Zhao & Tepper, 2007; Mattes, 2004), sourness (Prescott, Soo, Campbell & Roberts, 2004), astringency (Pickering et al. 2006; Pickering, Simunkova & DiBattista, 2004), saltiness (Sullivan et al. 2007), sweetness (Duffy, Peterson, Dinehart & Bartoshuk, 2003; Hayes & Duffy, 2007), and creaminess (Hayes et al. 2007; Duffy, Bartoshuk, Lucchina, Snyder & Tym, 1996; Tepper & Nurse, 1997) from foods and beverages more intensely than pMTs and/or pNTs. Hedonic responses also vary with perceived PROP intensity and PTS. pNTs are more likely to be sweet 'likers', those whose hedonic responses increase with increasing sweetness, while pSTs are more likely to be sweet 'dislikers', those whose hedonic responses decreased

with increasing sweetness (Looy & Weingarten, 1992; Yeomans, Tepper, Rietzschel & Prescott, 2007). PROP bitterness intensity, used as a continuous measure or in the categorization of individuals into PTS groups, has been shown to predict liking of cruciferous vegetables, coffee, grapefruit juice, high-fat foods, whiskey, and some beers when ratings are taken from sampled foods or self-reported checklists (Drewnowski et al. 1999a; Dinehart et al. 2006; Intranuovo et al 1998; Lanier et al. 2005; Drewnowski, Henderson, Ahlstrom, Clayton, Berg & Ruffin, 2000; Drewnowski, Henderson & Shore, 1997; Drewnowski, Henderson, Shore & Barratt-Fornell, 1998a; Duffy, Fast, Cohen & Bartoshuk, 1999; Tepper & Nurse, 1998; Villarino, Fernandez, Alday & Cubelo, 2009). It is hypothesized that pNTs' greater liking of high-fat foods leads them to consume more high-fat foods, which, over time, could lead to increased weight gain and obesity-related disease (Duffy, 2007; Tepper & Ullrich, 2002). Some studies have found that pNTs have a higher BMI and body fatness than pSTs (Goldstein, Daun & Tepper, 2005). The increased acceptance of alcohol-related sensations and the higher consumption of alcoholic beverages by pNTs could also contribute to an increased risk of disease and illness (Intranuovo et al. 1998; Guinard et al. 1996). pNTs have been found to present greater measured cardiovascular disease (CVD) risk (Duffy, 2004). The increased perception of bitterness in pSTs is associated with a reduced intake of vegetables (Dinehart et al. 2006), which are an important source of phytonutrients, and PROP intensity has been positively associated with a greater number of colonic polyps, a measure of colon cancer risk (Basson, Bartoshuk, Dichello, Panzini, Weiffenbach & Duffy, 2005). PROP's associations with food liking and consumption, and BMI are mediated by gender (Duffy et al. 1999; Tepper et al. 1998; Keller et al. 2009), dietary restraint (Tepper et al. 2002; Tepper, Koelliker, Zhao, Ullrich, Lanzara, d'Adamo, Ferrara, Ulivi, Esposito & Gasparini, 2008), and food adventurousness, a measure of neophobia (Ullrich, Touger-Decker, O'Sullivan-Maillet & Tepper, 2004).

Recently, Green and co-workers identified thermal taste as a new marker of individual variation in oral sensation (Cruz & Green, 2000). When a small area of the tongue is heated and/or cooled, thermal tasters (TTs), who constitute approximately 20-50% of the population sampled, perceive a phantom taste (Bajec et al. 2008a; Green & George, 2004a). Thermal sweetness is most likely to occur on the tongue tip when it is

re-warmed from an initial cooling period, thermal saltiness is sometimes reported upon cooling the same area, and thermal sourness is elicited in some individuals when the lateral edge of the tongue is cooled (Cruz et al. 2000). An examination of *Trpm5* knockout mice strongly suggests that TRPM5, a TRP superfamily cation channel with a role in the transduction of umami, sweet and bitter tastes (Zhang et al. 2003), is a component in the phenomenon of sweet thermal taste (Talavera, Yasumatsu, Voets, Droogmans, Shigemura, Ninomiya, Margolskee, & Nilius, 2005), presenting the possibility that that this index of individual variation is under genetic control. It has further been suggested that other tastes perceived by TTs with thermal stimulation (i.e., bitter, salty, sour) may be due to the temperature sensitivity of the channels involved in their chemical transduction (Talavera, Ninomiya, Winkel, Voets, & Nilius, 2007). TTs also rate salt, citric acid, quinine, PROP, and monosodium glutamate applied to the tongue tip, as well as whole-mouth rinses of sucrose, citric acid, and PROP, as more intense than thermal non-tasters (TnTs) (Bajec et al. 2008a; Green et al. 2004a). Retronasally and orthonasally presented vanillin (Green et al. 2004a), and metallic flavour (Bajec et al. 2008a) were also rated as more intense by TTs, which may suggest that the heightened responsiveness to oral and olfactory stimuli results from differences in gustatory and olfactory brain region excitability (Green et al. 2004a). While the burning, stinging and prickling sensations from capsaicin and menthol do not differ between TTs and TnTs (Green, Alvarez-Reeves, George & Akirav, 2005), TTs do perceive low and high levels of astringency with greater intensity than TnTs (Bajec et al. 2008a). Unlike PTS groups (Bajec et al. 2008a; Bartoshuk et al. 1994; Tepper et al. 1997; Reedy et al., 1993), TTS groups do not differ in fungiform papillae (FP) density, and PTS and thermal taster status (TTS) do not interact for intensity ratings of orosensory stimuli, suggesting that PTS and TTS function via independent mechanisms (Bajec et al. 2008a).

Given the role of genetics in taste perception and food selection (reviewed in: Duffy 2007; Garcia-Bailo et al. 2009), and its overall importance in eating behaviours (de Krom, Bauer, Collier, Adan & la Fleur, 2009), it is difficult to imagine that only one index of individual variation in taste is associated with food liking. The primary objective of the current study was to examine the influence of TTS on food liking, BMI,

and WC. A secondary objective was to reassess the influence of PTS on these variables, and to investigate the relationship between TTS and PTS on food liking.

Methods

Subjects

132 subjects were recruited from the student, staff, and faculty populations of Brock University, and from the local community. Incentive was provided in the form of a monetary prize or credit toward a 1st year university Psychology course. 5 subjects were removed from the dataset due to missing data. The final cohort consisted of 84 females and 43 males with a mean age of 31 years±11SD (range: 18 to 68). To establish ethnic origin, the Census Canada "Ethnic Origin User Guide" (Statistics Canada, 2001) was employed. 105 subjects were Caucasian (reporting 'White' as their ethnicity; 34 males), and the remaining 22 were non-Caucasian (9 males). 12 subjects reported that they smoked: 5 females, and 7 males. The Brock University Research Ethics Board approved all procedures, and written consent was obtained from all subjects.

Scale

Paper versions of the general Labeled Magnitude Scale (gLMS) were used to collect all PROP intensity and thermal taste intensity ratings (Bartoshuk, Duffy, Fast, Green, Prutkin & Snyder, 2002; Bartoshuk et al. 2004). Subjects received verbal and written instructions that the top of the scale represented the most intense sensation in any modality that they could ever imagine experiencing, and were told to think of experiences from a variety of different modalities to assist in understanding the general nature of the scale (Bartoshuk et al. 2002). In order to familiarize subjects with the gLMS and facilitate correct scale use, they were asked to rate the intensities of 5 remembered sensations: sourness of a lemon, pain from biting your tongue, coolness of an ice-cold beverage, burning sensation from eating a whole hot pepper, and brightness of the sun when looking directly at it (Green & Hayes, 2004b; Porubcan & Vickers, 2005).

6-n-propylthiouracil (PROP)

Subjects rinsed with a 20 ml volume of 0.32 mM 6-n-propylthiouracil (PROP; MP Biomedicals; OH, USA) solution, or as much as physically possible, for 10 s, expectorated, and waited for the bitterness intensity to peak (on average 10-15 s) before providing a rating (Bajec et al. 2008a).

Thermal Taste (TT)

A 64 mm^2 computer-controlled Peltier device with a thermocouple feedback attached to a toothbrush-sized water-circulated heat sink (thermode) was applied to the subject's extended tongue by the researcher. Subjects were instructed to rate the intensity of all oral sensations, including temperature, that they perceived in each trial. Three locations on the edge of the tongue were stimulated discretely and in order: the most anterior tip, and approximately 1 cm to the right and then the left of the midline. Warming trials started at 35°C, cooled to 15°C, and re-warmed to 40°C (held for 1 s). The start temperature for cooling trials was 35°C, followed by cooling to 5°C (held for 10 s). Warming trials preceded cooling trials at each location to avoid possible adaptation from the intense, sustained cold stimulation (Green et al. 2004a), and all warming trials (tip, right, left) were performed before all cooling trials (Bajec et al. 2008a).

Food Liking and Food Groups

Subjects completed questionnaires including alcohol liking and consumption measures, and food and non-alcoholic beverage (herein referred to as food(s) or food items) liking measures. The food item list was loosely based on Meiselman and Waterman (1978). The 332-item list included different preparations of foods (e.g., roast beef, beef steak, beef stew; steamed rice, risotto, fried rice), and included prepared foods, raw foods, and in some cases preserved foods. Subjects rated their liking of food items on a 7-point Likert scale (Lawless & Heymann, 1998) ranging from 'like extremely' to 'dislike extremely'. Subjects could also indicate allergy, lack of exposure to the food, and lack of knowledge of the food in lieu of providing a liking rating.

To examine PTS effects on cruciferous vegetable, grapefruit, and bitter beverage liking, these items were examined independently. In a preliminary investigation of TTS

effects on food liking, one-way ANOVAs were performed on all food items individually. Additionally, three separate food groupings were created: Food Groups, Orosensory Groups, and Correlation Groups. Food Groups were based on food type (Villarino et al. 2009; Table 1), which included the main groups (and sub-groups) *Dairy* (yogurt, milk, frozen/sweet dairy, cream), *Meat* (poultry, beef, pork, hamburger/hot dog/sausage, fish, seafood, other), *Grain* (rice, pasta, breads, hot cereal, cold cereal, crackers), *Vegetable* (cooked vegetables, raw vegetables), *Fruit* (cooked fruits, raw fruits, preserved fruits), *Dessert* (cookies, bars, cakes, donuts, pies, pudding, other), *Eggs*, *Nuts*, *Curry*, *Horseradish*, and *Wasabi*. Orosensory Groups were created through the identification and grouping of a sub-set of foods that share a predominant taste, texture, or chemesthetic quality. The Orosensory Groups examined were Sweet (ice cream, chocolate milk, milk shake, raw apricot, raw banana, brownies, cheesecake, iced cake, cookies, donuts, gelatin, fruit crisp, pies, flavoured pudding), Bitter (espresso, black coffee, tonic water, raw and cooked broccoli, raw/cooked cauliflower, raw radish, raw endive, raw and cooked spinach, grapefruit, cooked Brussels sprouts), Salty/Savoury (cheeses, beef, chicken, fish, hotdogs, hamburgers, sausage, lamb, pork, shell fish, shrimp, breadsticks, bread stuffing, crackers, croissants), Hot (curry, wasabi, horseradish, hot peppers), Mushy (soft cheeses, hot cereal (oat and wheat), cooked tofu, creamed corn, raw and cooked mushroom, cooked peas, cooked squash, cooked turnip, cooked zucchini, cooked apples, raw avocado, raw banana), and Fatty (cheeses, creams, milks, ice cream, fried chicken, eggs, fried fish, hamburgers, hotdogs, sausage, lamb roast, bacon, beef, onion rings, mashed potatoes, fried potatoes, brownies, cheese cake, iced cake, cookies, donuts, pie, flavoured pudding). Correlation Groups were created for each phenotype separately by grouping foods that were correlated (Pearson's r) with either PTS or TTS at the $p<0.1$ level (Duffy, Lucchina & Bartoshuk, 2004c). PROP intensity was used as a continuous variable to determine its association (Pearson's r) with food items, and point biserial correlations were performed between food items and TTS. Correlated foods were noted and groups were created based on the direction of the association and the underlying similarities between the food items. Correlation Groups for PROP included Fat (homogenized milk, buttermilk, hotdogs, pork chops, cream, onion rings, pasta salad), Non-cruciferous Vegetables (raw and cooked beans, creamed

Table 1. Individual food and beverage items that compose the Food Groups and sub-groups, Orosensory Groups, and TTS and PTS Correlation Groups.

Groupings	***Groups*** **Sub-groups**	**Food/beverage Items**
Food Groups	*Dairy*	All Dairy
	Yogurt	Flavoured, Plain
	Milk	1%, 2%, Skim, Homogenized, Butter, Chocolate
	Frozen/sweet dairy	Whipped cream, Ice cream, Milk shake (any flavour)
	Cream	10%, Table, Sour
	Meat	All Meat
	Poultry	Fried, Grilled, Roasted
	Beef	Ribs, Roast, Steak, Stew, Veal
	Pork	Bacon, Chop, Ham
	Hamburger/Hot dog/Sausage	Hamburger, Hot dog, Sausage
	Fish	Baked, Broiled/steamed, Fried, Stew
	Seafood	Shellfish boiled/steamed, fried, grilled; Shrimp boiled/steamed, cocktail, fried
	Other	Ostrich, Emu; Venison, Moose; Goat roast, stew; Lamb roast, stew; Organ meat fried, pie, roasted
	Grain	All Grain
	Rice	Cakes, Fried, Noodles, Steamed, Risotto
	Pasta	Salad, Plain, Sauce, Stuffed
	Breads	Brown/wheat, Grain/seed, Pumpernickel, Sourdough, Sticks, Stuffing, White, Cornbread stuffing, Croissant
	Hot cereal	Corn, Oat, Rice, Wheat, Barley, Buckwheat, Cornmeal
	Cold cereal	Corn, Oat, Rice, Wheat
	Crackers	Flavoured, Plain, Salted

	Vegetable	All Vegetables
	Raw	Arugula, Asparagus, Bean, Beet, Bok Choy, Broccoli, Brussels Sprouts, Cabbage, Carrots, Cauliflower, Celery, Cucumber, Eggplant, Endive, Kale, Kohlrabi, Leek, Lettuce, Mushroom, Mustard greens, Okra, Onion, Green peas, Bell peppers, Hot peppers
	Cooked	All Raw +Boiled corn, Creamed corn, Potato boiled, Potato baked, Potato fried, Potato salad, Collard greens, Onion rings (fried)
	Fruit	All Fruits
	Cooked fruits	Apple, Apricot, Avocado, Banana, Blueberry, Blackberry, Sweet cherry, Sour cherry, Currant, Fig, Grapefruit, Guava, Kiwi, Lychee, Mango, Melon, Olive, Oranges, Papaya, Peaches, Pear, Pineapple, Plantain, Plum, Raspberry, Strawberry
	Raw fruits	Cooked fruits + Grapes, Pomegranate
	Preserved fruits	Cooked fruits + Grapes, Pomegranate
	Dessert	All Desserts
	Cookies	Decorated, Fruit, Plain, Soft, Spiced
	Bars	Fruit, Nut
	Cakes	Cheese, Fruit, Iced, Plain, Spice
	Donuts	Plain, Fancy
	Pies	Cream, Deep fried, Fruit, Nut, Pudding, Pumpkin
	Pudding	Fruit, Rice, Flavoured
	Other	Fruit crisp, Fruit/flavoured gelatin, Fruit strudel
	Eggs	Boiled, Fried, Poached, Prepared, Creamy salad, Salad
	Nuts	Almond, Beechnut, Cashew, Chestnut, Coconut, Hazel nut, Pecan, Peanut, Pine nut, Pistachio, Walnut

	Curry	Mild, Medium, Hot
	Horseradish	Mild, Medium, Hot
	Wasabi	Mild, Hot
Orosensory Groups	Sweet	Ice cream, Chocolate milk, Milk shakes, Raw apricot, Raw banana, Brownies, Cheesecake, Iced cake, Cookies (all), Donuts (all), Gelatin, Fruit crisp, Pies (all), Flavoured pudding
	Bitter	Espresso, Black coffee, Tonic water, Raw & cooked broccoli, Raw & cooked cauliflower, Raw radish, Raw endive, Raw & cooked spinach, Grapefruit, Cooked Brussels sprouts
	Salty/Savoury	Cheeses (all), Beef, (all), Chicken (all), Fish (all), Hotdogs, Hamburgers, Sausage, Lamb (all), Pork (all), Shell fish (all), Shrimp (all), Breadsticks, Bread stuffing, Crackers, Croissants
	Hot	Curry (hot), Wasabi (hot), Horseradish (hot), Hot peppers
	Mushy	Soft cheese, Hot cereal (oat and wheat), Cooked tofu, Creamed corn, Raw & cooked mushroom, Cooked peas, Cooked squash, Cooked turnip, Cooked zucchini, Cooked apples, Raw avocado, Raw banana
	Fatty	Cheeses (all), Creams (all), Homogenized milk, Butter milk, Milk shake, Ice cream, Fried chicken, Egg (all), Fried fish, Hamburgers, Hotdogs, Sausage, Lamb roast, Bacon, Beef (all), Onion rings, Potatoes mashed, Potatoes fried, Brownies, Cheese cake, Iced cake, Cookies (all), Donuts (all), Pie (all), flavoured pudding
TTS Correlation	Fruit/Vegetables	Cooked and preserved apples, Cooked banana, Cooked and preserved blueberry, Cooked sweet

Groups	Fruit/Vegetables	cherry, Cooked kiwi, Cooked and preserved mango, Cooked peaches, Cooked and preserved plums, Cooked and preserved raspberry, Cooked peas, Cooked zucchini
	Fruit	Raw plums, Raw blueberries, Raw sweet cherry, Raw mango, Raw tomato
	Bitter	Espresso, Cooked turnip, Cooked rutabaga, Cooked mustard greens, Cooked collard greens
PROP Correlation Groups	Fat	Homogenized milk, Butter milk, Hotdogs, Pork chops, 10% cream, Table cream, Onion rings, Creamy Pasta salad
	Non-cruciferous vegetables	Raw & cooked beans, Creamed corn, Raw and cooked leeks, Cooked endive
	Bitter	Espresso, Cooked rutabaga, Cooked mustard greens, Raw and cooked kohlrabi, Raw Brussels sprouts
	Fruit	Raw and cooked sweet cherry, Preserved sour cherry, Preserved currant, Raw and preserved figs, Raw and cooked and preserved papaya, Raw and preserved plums, Preserved and cooked oranges, Preserved raspberries

corn, raw and cooked leeks, cooked endive), Bitter (espresso, cooked rutabaga, cooked mustard greens, raw and cooked kohlrabi, raw Brussels sprouts), and Fruit (raw and cooked sweet cherry, preserved sour cherry, preserved currant, raw and preserved figs, raw and cooked and preserved papaya, raw and preserved plums, preserved and cooked oranges, preserved raspberries). TTS Correlation Groups included Bitter (espresso, cooked turnip, cooked rutabaga, cooked mustard greens, cooked collard greens), Fruit (raw plums, raw blueberries, raw sweet cherry, raw mango, raw tomato), Fruit/Vegetable (cooked and preserved apples, cooked banana, cooked and preserved blueberry, cooked sweet cherry, cooked kiwi, cooked and preserved mango, cooked peaches, cooked and preserved plums, cooked and preserved raspberry, cooked peas, cooked zucchini). For both Orosensory Groups and Correlation Groups, Cronbach's α was used to ensure internal reliability of the groups, with each group having $\alpha > 0.7$ (Drewnowski et al. 1998a; Duffy et al. 2004c; Duffy & Bartoshuk, 2000).

Anthropometric Measures

Anthropometric measures were taken after Goldstein et al. (2005). Weight was measured to the nearest 0.25 kg using an analog scale (Zenith, New Castle, DE, USA), and height was measured to the nearest 0.1 cm using a meterstick (Mastercraft; Canadian Tire, ON, Canada) affixed to a flat, straight wall. Height and weight were used to calculate body mass index (BMI; kg/m^2). Waist circumference (WC; cm) was measured between the lowest rib and the iliac crest to the nearest 0.1 cm with the subjects in the standing position using a tape measure (Robard Corporation, Mt Laurel, NJ, USA). All measures were taken over light clothing and without shoes.

Neophobia

In addition to 2 novel questions, a subset of 8 questions from Pliner and Hobden's (1992) Food Neophobia Scale were used to determine subjects' reluctance and/or avoidance of novel foods. The specific questions taken from Pliner et al. (1992) were: I am constantly sampling new and different foods/beverages; I don't trust new foods/beverages; I like foods/beverages from different countries; Ethnic foods/beverages look too weird to eat/drink; At dinner parties, I will try a new food; I am afraid to eat/drink things I have never had before; I will eat almost anything; and, I

like to try new ethnic restaurants. The additional, novel questions were: I initiate trying new foods, and I will try new foods/beverages when I am alone, both of which were significantly correlated (r=0.77, P=2E-25 and r=0.76, P=7E-18, respectively) with the total score of the 8 Pliner et al. (1992) questions. The total score of all 10 questions was significantly correlated with the total score of the 8 Pliner et al. (1992) questions (r=0.98, p=2E-94). For inclusion as an ANOVA factor, two categories were created using the neophobia measure; neophobes were defined as those whose overall neophobia score was greater than or equal to 35, and non-neophobes were those with neophobia scores under 35. A score of 35 was chosen as the cut-off for this categorization because it represents half of the maximum possible score (70) on the neophobia questionnaire.

PROP Taster Status (PTS) and Thermal Taster Status (TTS) Categorization

Duplicate PROP intensity ratings were averaged and PTS groups were defined as: pNTs<10.9 mm; pMTs 10.9-61.5 mm; and pSTs >61.5 mm (Porubcan et al. 2005). In order to compare the perceived intensity of PROP across individuals, data were rescaled relative to a non-taste sensation (Bartoshuk et al. 2002). The remembered intensity of "brightness of the sun when looking directly at it" was used to standardize the data (Bartoshuk et al. 2002; Porubcan et al. 2005). Each subject's brightness rating was divided by the group average for this remembered sensation, creating an individualized normalization factor by which PROP ratings were divided.

In order to maintain the position of "weak" on the gLMS, thermal taste data were not normalized. TTs were defined as those that reported the same taste sensation, rated above weak, at the same location and temperature in both replicates (Green et al. 2004a). Those that did not perceive any taste sensations in any trial were defined as TnTs.

Statistical Analysis

All analyses were performed with SPSS 16 (SPSS Inc., IL, USA). Univariate outliers were defined as having a standardized Z-score >/= |3.29| (Tabachnick & Fidell, 2006). Skewed variables, defined as those having a skew or kurtosis of greater than ±2, were transformed (square root or log) to improve distribution normality for all statistical

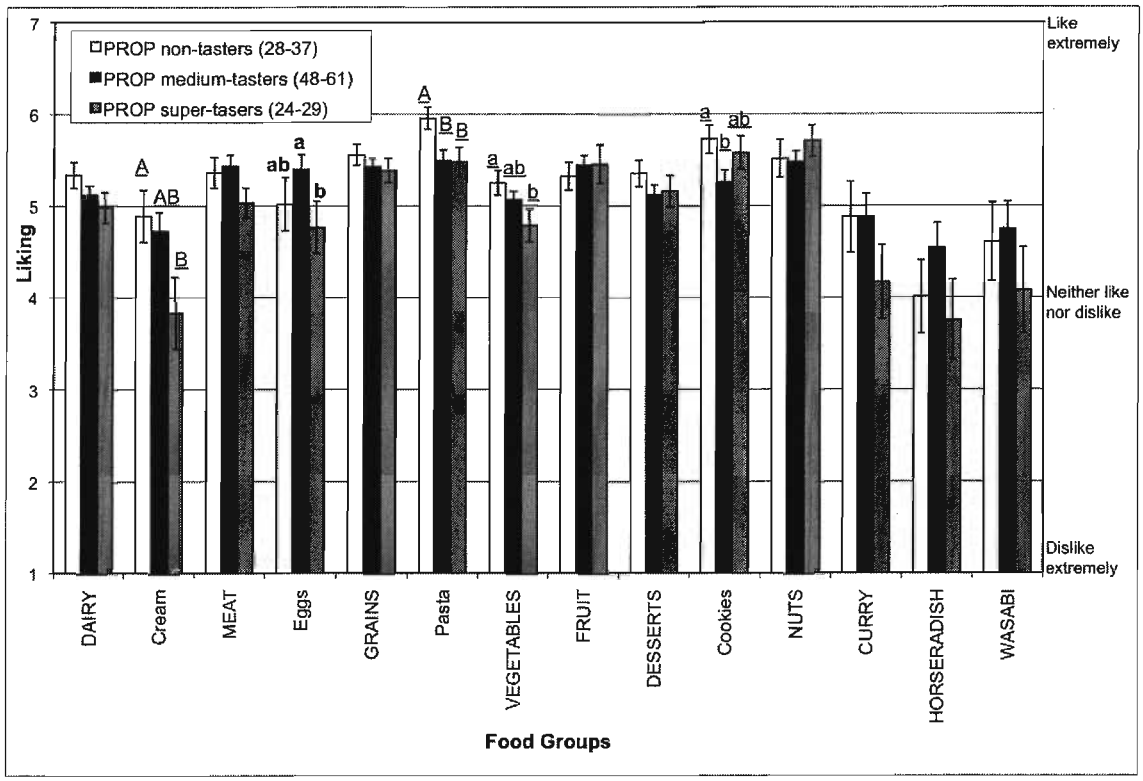
analyses (Tabachnick et al. 2006). Three-way ANOVA examining the effects and interactions of PTS and TTS with gender and neophobia was performed on liking scores for Food Groups, Orosensory Groups, and Correlation Groups. Where three-way effects and/or interactions were not significant, one-way ANOVA was employed to examine effects of PTS and TTS independently. Where applicable, Tukey's HSD was used as the mean separation test following a significant one- or three-way ANOVA. The perceived intensity of PROP bitterness was treated as a continuous variable allowing for examination of its association (Pearson's r) with neophobia, BMI, and waist circumference.

Results

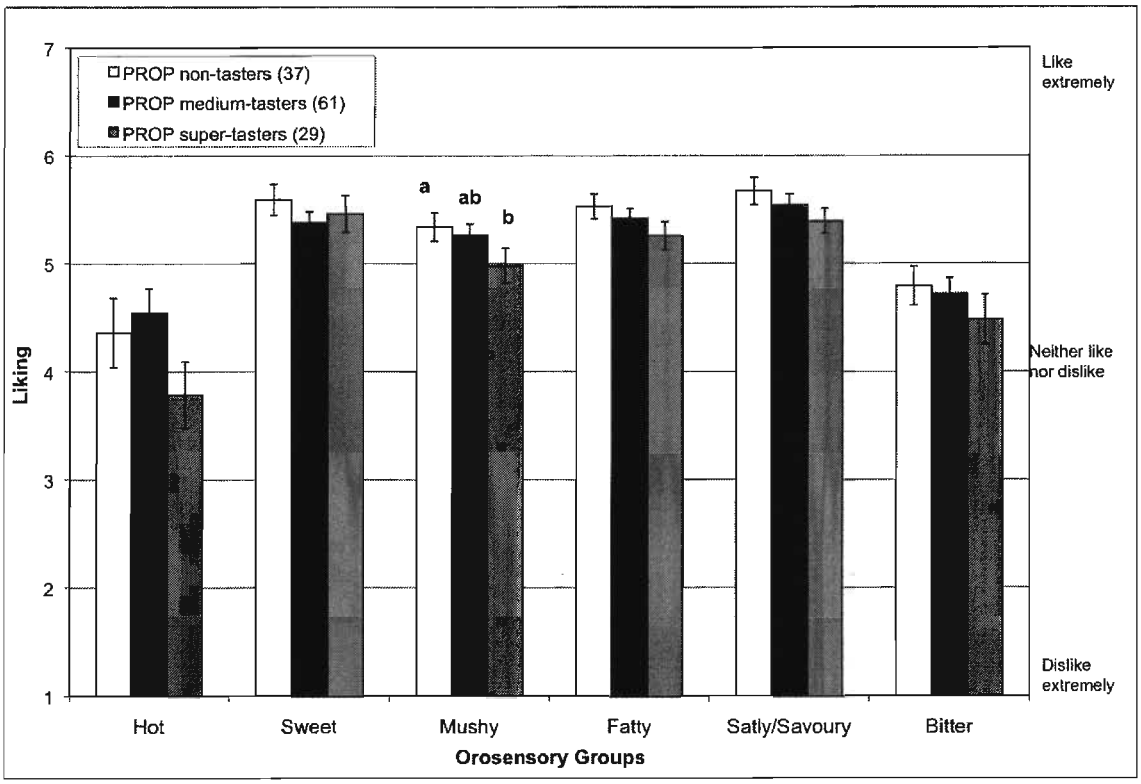
TTS categorization yielded 26 TTs (10 males, 5 non-Caucasian, 0 smokers), and 50 TnTs (14 males, 6 non-Caucasian, 5 smokers). 51 subjects could not be categorized for TTS. PTS categorization yielded 37 pNTs (13 males, 6 non-Caucasian, 5 smokers), 61 pMTs (22 males, 14 non-Caucasian, 6 smokers), and 29 pSTs (8 males, 2 non-Caucasian, 1 smoker). The average neophobia score was 23.8±12.2SD, and 22 subjects were categorized as neophobes and 104 as non-neophobes. Average BMI was 25.7±5.2SD, 5 subjects were underweight (BMI<18.5), 58 were of normal weight ($18.5 \leq BMI < 25$), 42 were overweight ($25 \leq BMI < 30$), and 22 were obese ($BMI \geq 30$). BMI did not differ between men and women ($t=0.92$, $P=0.36$). Analyses and discussion of psychophysical and physiological measures examined in this subject cohort are presented in Bajec et al. (2008a). Means and standard errors for TTS and PTS on Food Group, Orosensory Group, and Correlation Group liking are summarized in Figures 1 and 2, respectively. Significant PTS*gender and PTS*neophobia interactions are presented in Figures 3 and 4, respectively. Throughout the text, where applicable, means are presented with (±) standard errors unless otherwise noted.

TTS

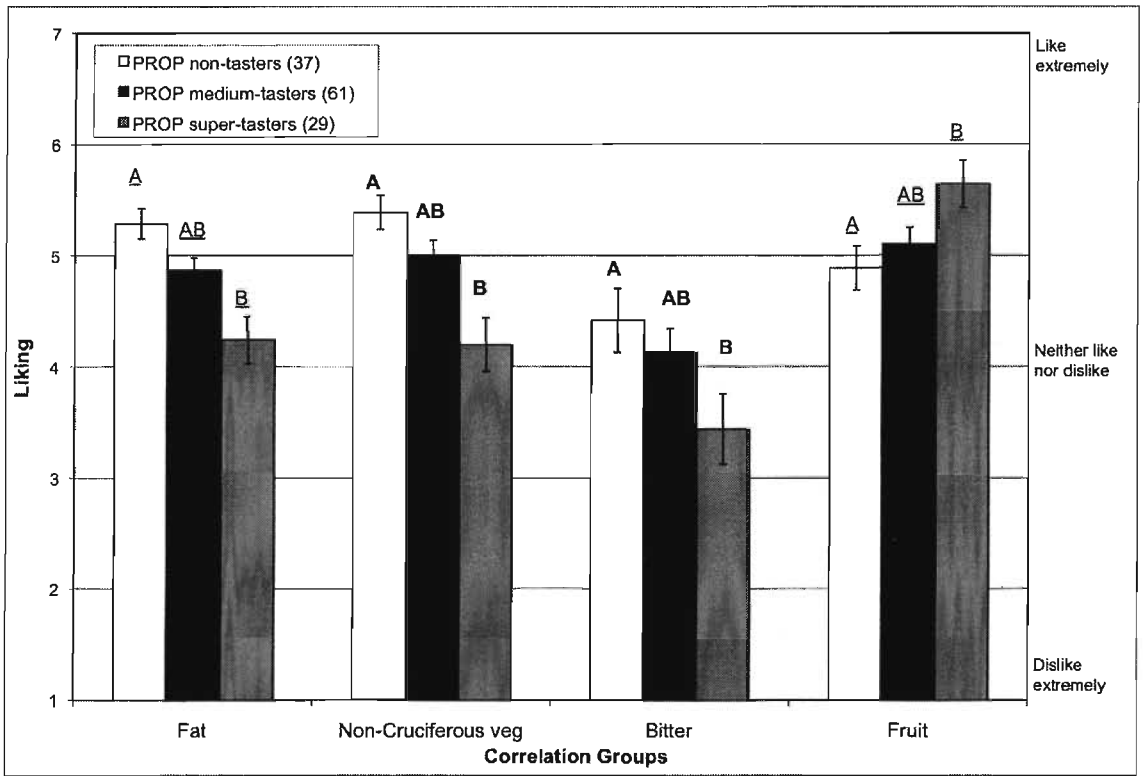
TTs gave significantly lower liking scores for the Cooked Fruit Food Group ($F(1,75)=5.67$, $p<0.05$), and the Cooked Fruit/Vegetable ($F(1,75)=8.79$, $p<0.01$) and Bitter ($F(1,74=9.50$, $p<0.01$) Correlation Groups. Differences between TTS groups'


PROP non-tasters (28-37)
PROP medium-tasters (48-61)
PROP super-tasers (24-29)
Liking
Like extremely
Neither like nor dislike
Dislike extremely
DAIRY
Cream
MEAT
Eggs
GRAINS
Pasta
VEGETABLES
FRUIT
DESSERTS
Cookies
NUTS
CURRY
HORSERADISH
WASABI
Food Groups

A)

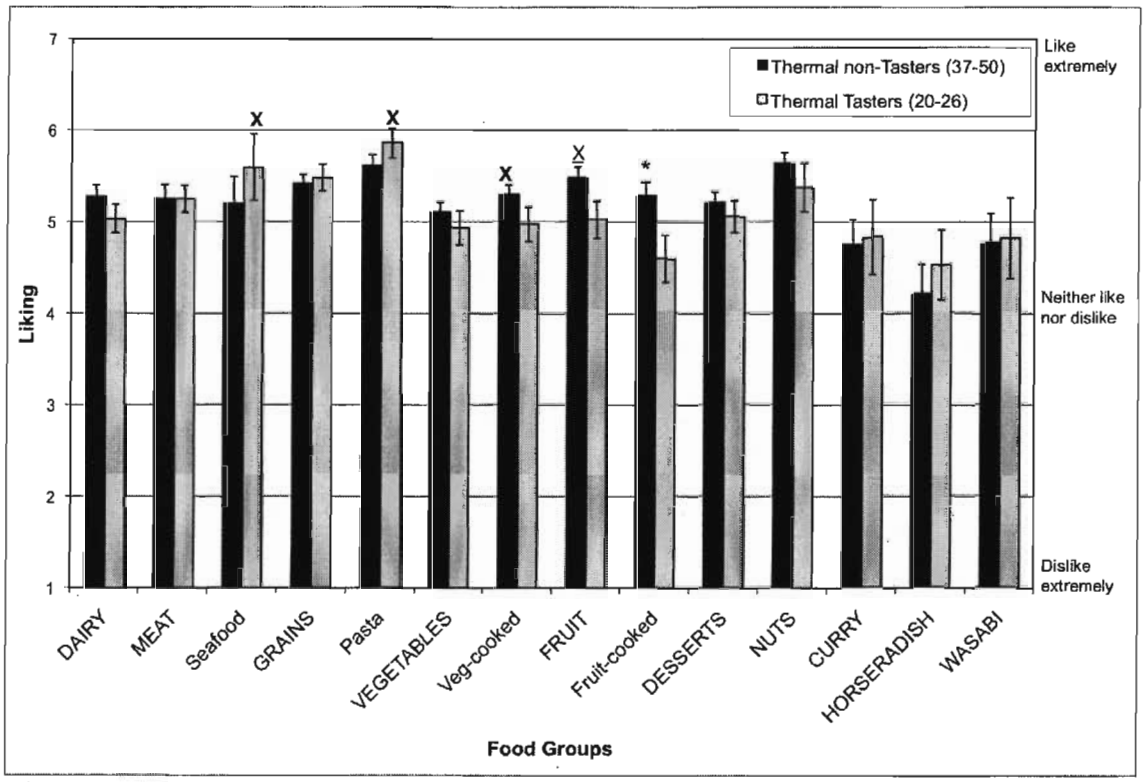

PROP non-tasters (37)
PROP medium-tasters (61)
PROP super-tasers (29)
Liking
Like extremely
Neither like nor dislike
Dislike extremely
Hot
Sweet
Mushy
Fatty
Satly/Savoury
Bitter
Orosensory Groups

B)

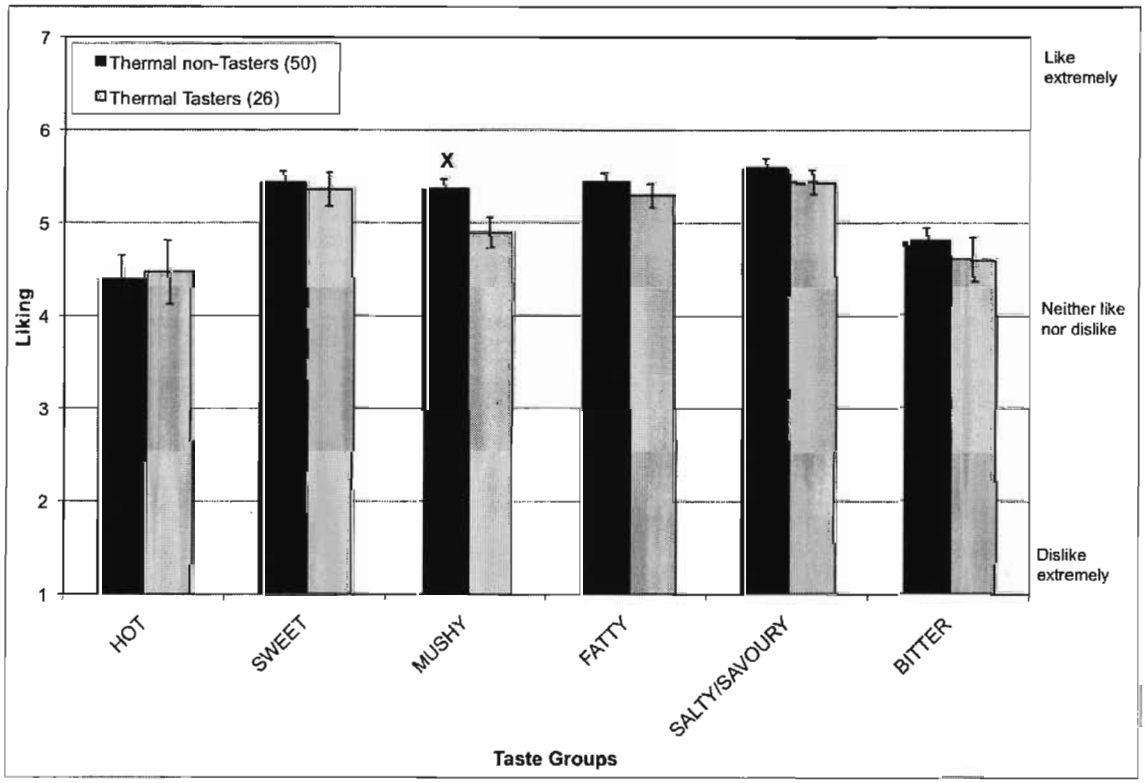


C)

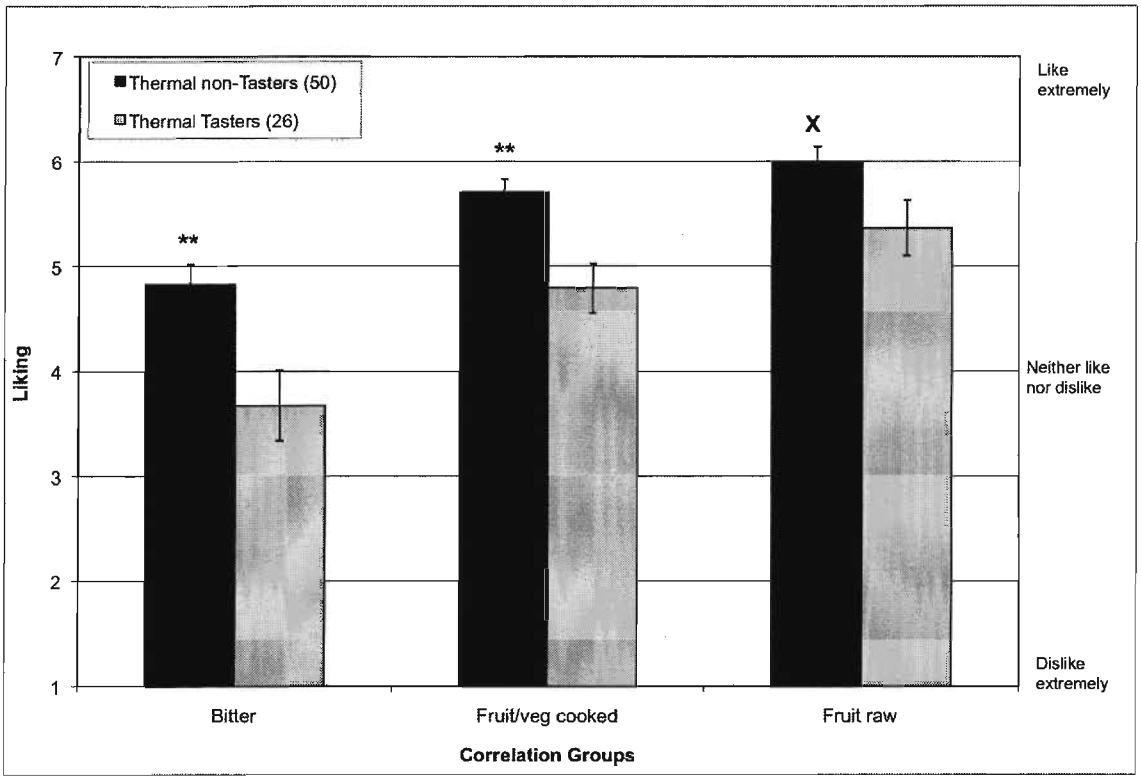
Figure 1. PROP taster status (PTS) effects on liking of A) Food Groups (uppercase) and subgroups (lowercase), B) Orosensory Groups, and C) Correlation Groups. Bars represent mean liking scores ±SE mean. Means with different letters differ significantly at the $p<0.1$ level (lowercase letters) or the $p<0.05$ level (uppercase letters) using either 1-way ANOVA (underlined) or 3-way ANOVA (bold).


Thermal non-Tasters (37-50)
Thermal Tasters (20-26)
Like extremely
Neither like nor dislike
Dislike extremely
Liking
7
6
5
4
3
2
1
X
X
X
X
*
DAIRY
MEAT
Seafood
GRAINS
Pasta
VEGETABLES
Veg-cooked
FRUIT
Fruit-cooked
DESSERTS
NUTS
CURRY
HORSERADISH
WASABI
Food Groups

A)

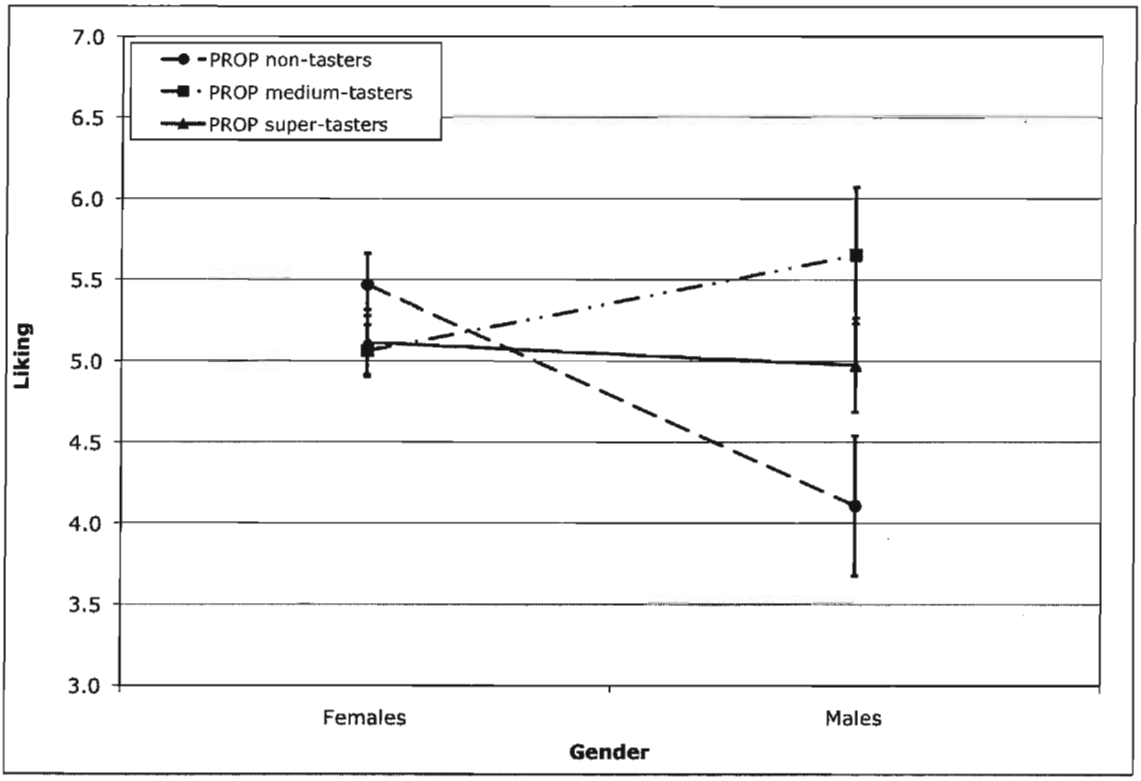

Thermal non-Tasters (50)
Thermal Tasters (26)
Like extremely
Neither like nor dislike
Dislike extremely
Liking
7
6
5
4
3
2
1
X
HOT
SWEET
MUSHY
FATTY
SALTY/SAVOURY
BITTER
Taste Groups

B)_

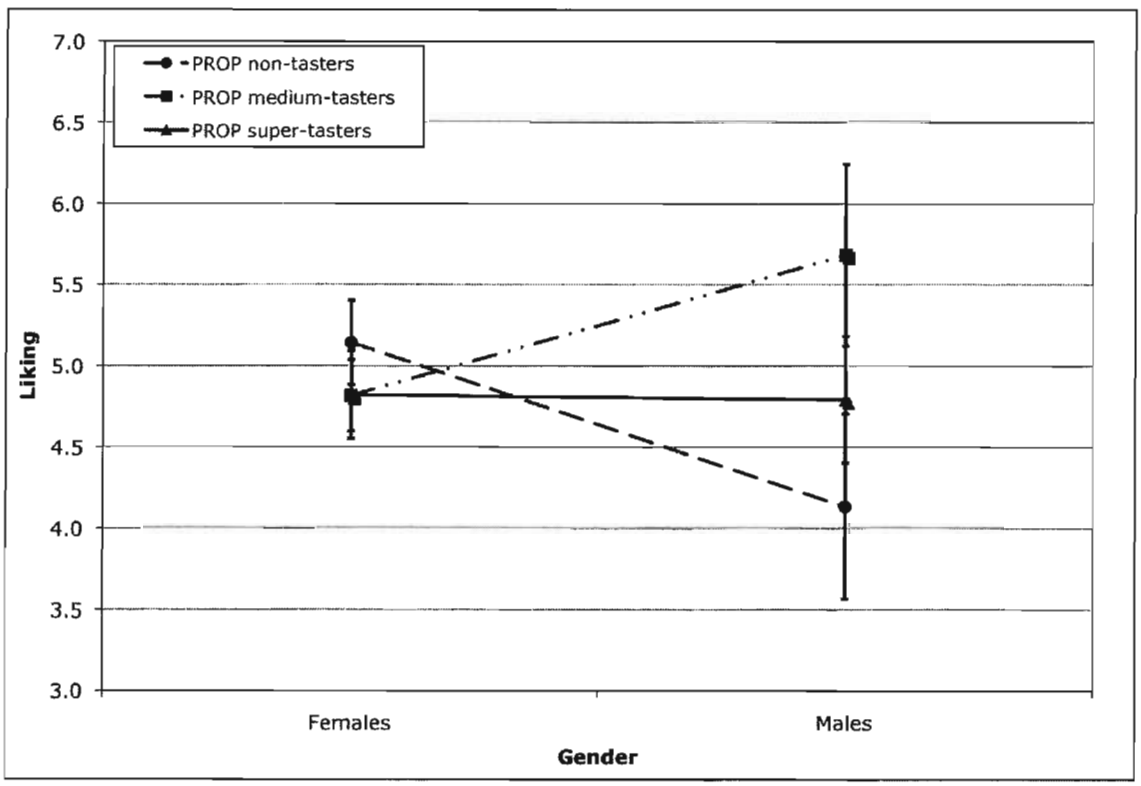


C)

Figure 2. Thermal taster status (TTS) effects on liking of A) Food Groups and subgroups, B) Orosensory Groups, and C) Correlation Groups. Bars represent mean liking scores ±SE mean. Means that differ at significance levels of $p<0.01$, $p<0.05$, and $p<0.1$ are indicated by **, *, X, respectively. Analysis was performed using either 1-way ANOVA (underlined) or 3-way ANOVA (bold).


7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
Liking
PROP non-tasters
PROP medium-tasters
PROP super-tasters
Females
Males
Gender

A)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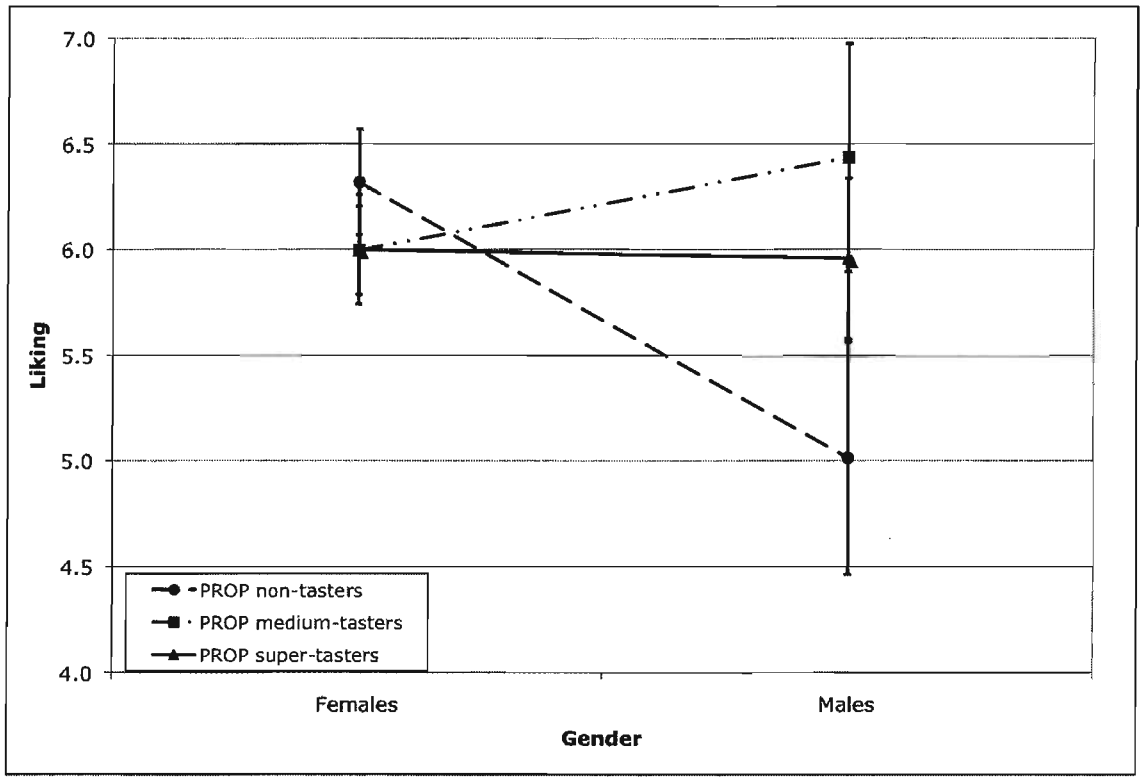
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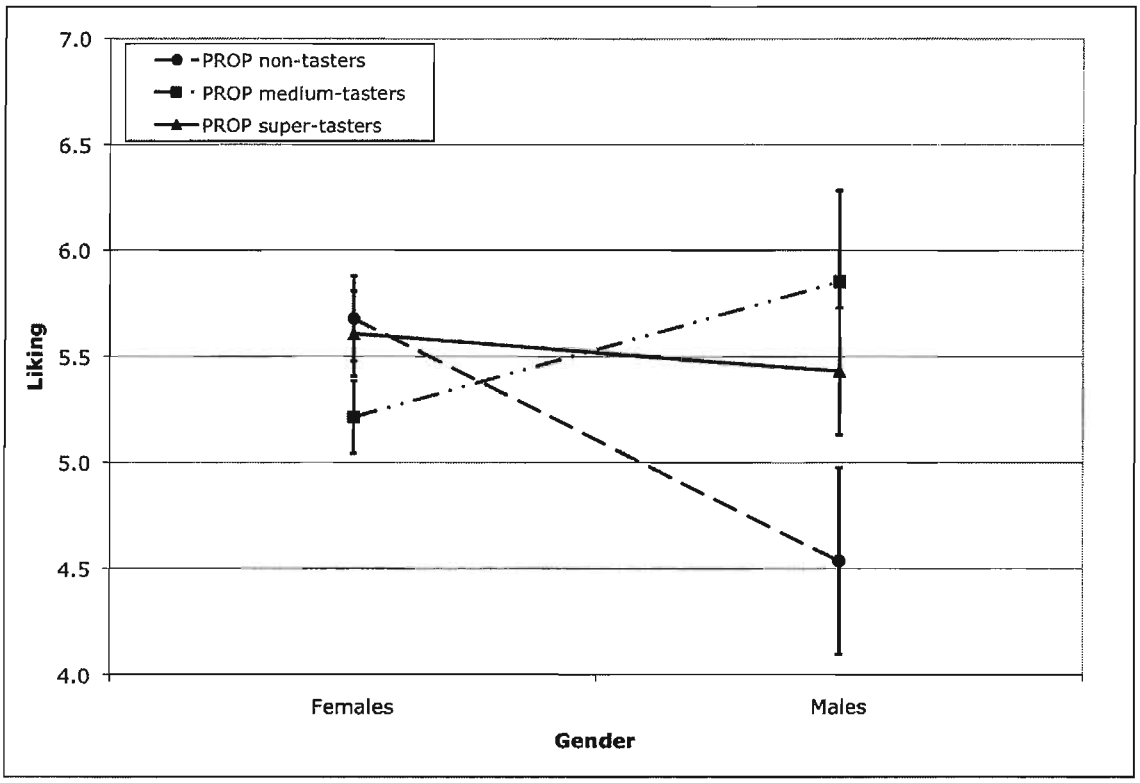
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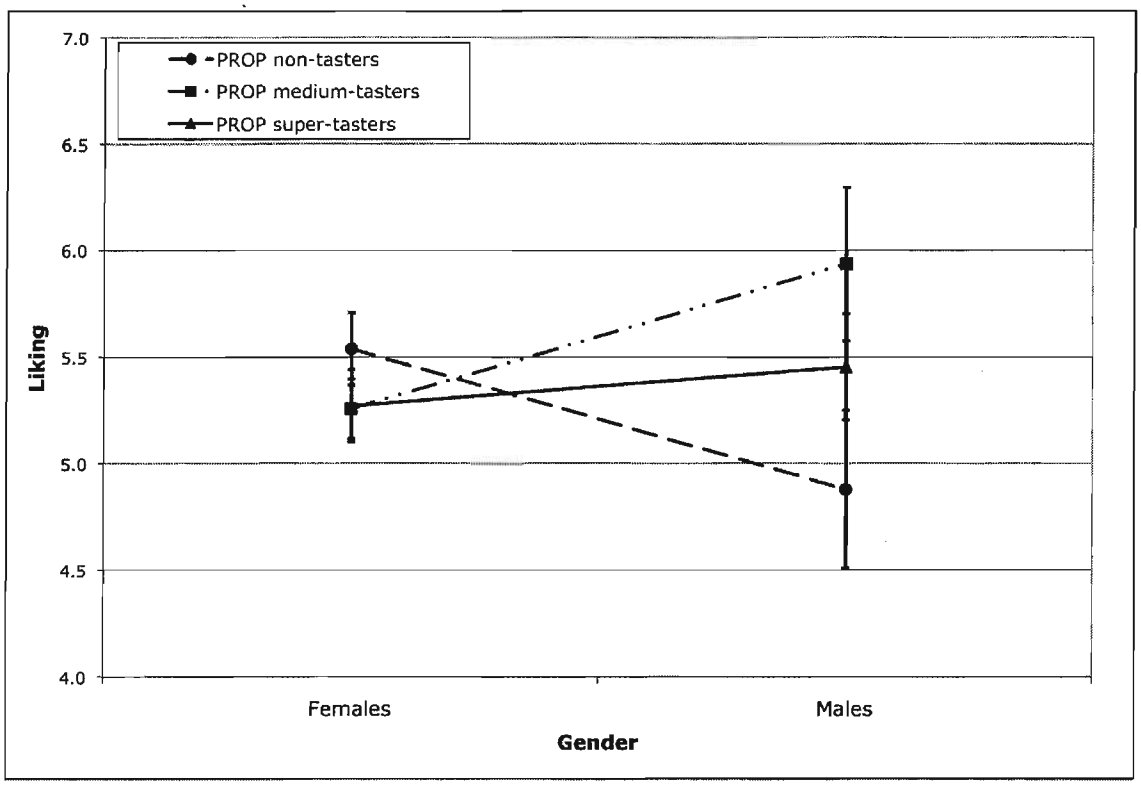
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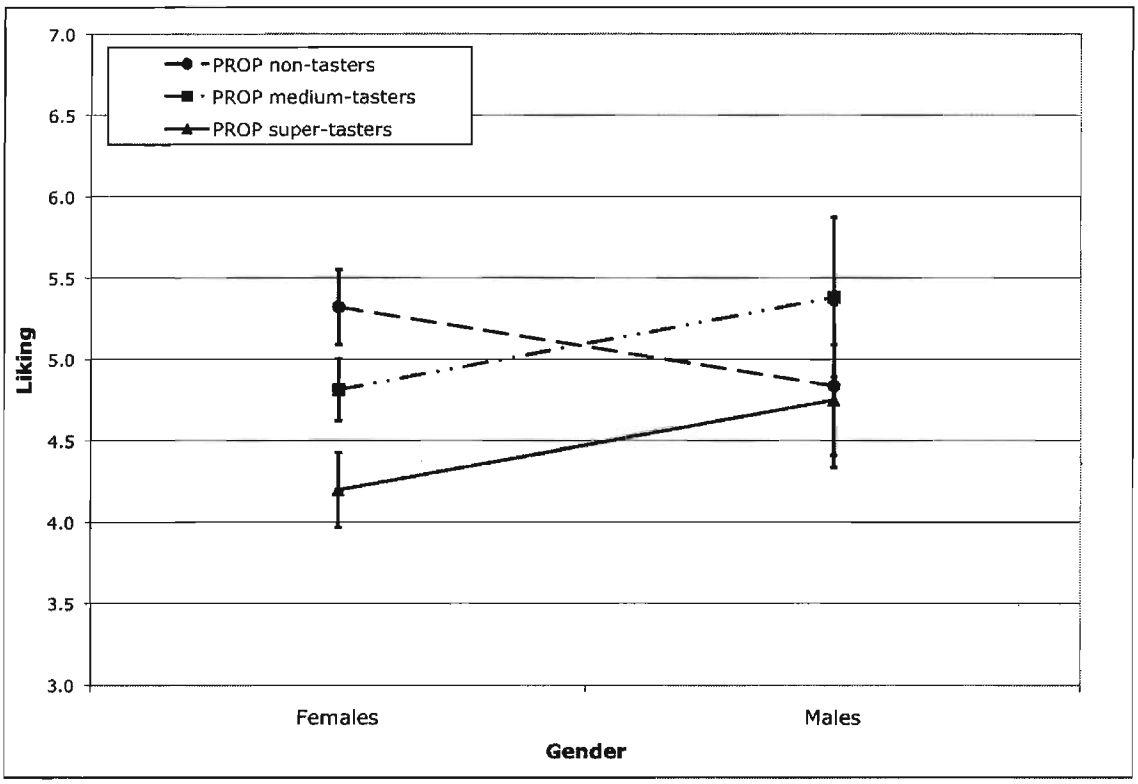
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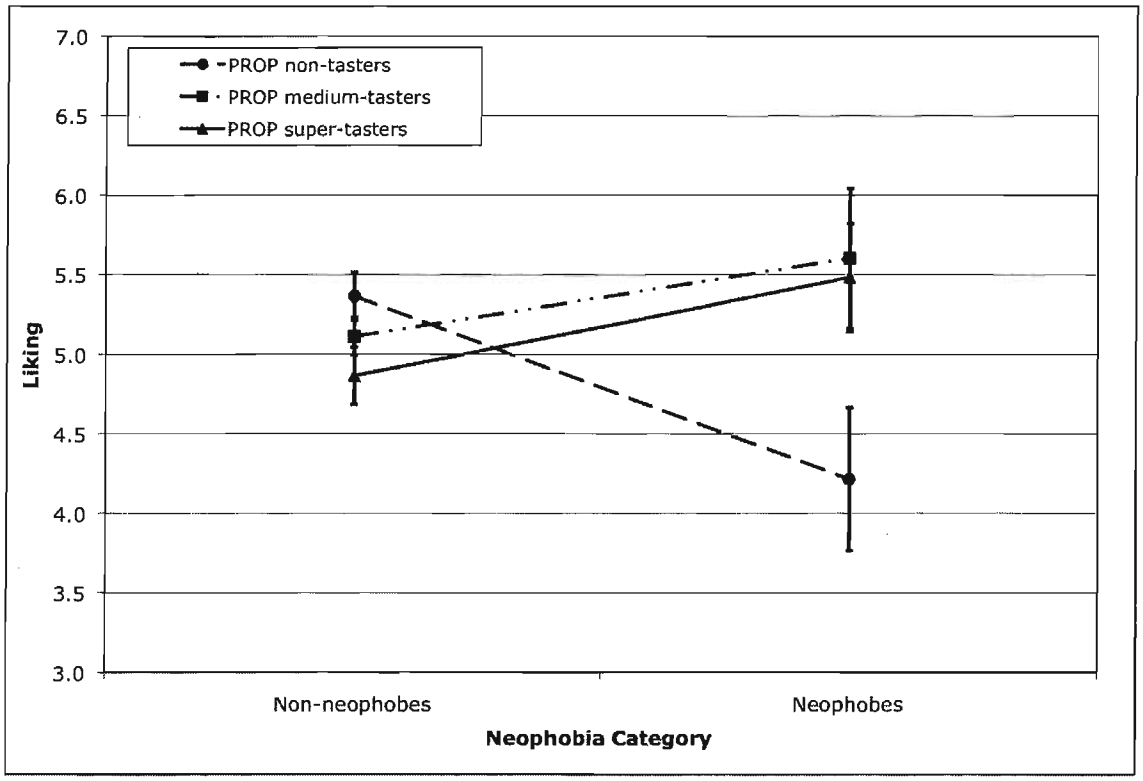
F)

Figure 3. PROP taster status (PTS)*gender interaction for the A) Dairy Food Group and B) Milk and C) Frozen/sweet sub-groups, the D) Sweet and E) Fatty Orosensory Groups, and F) the Fat Correlation Group. Line symbols represent mean liking ratings ±SE mean.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Neophobia Category

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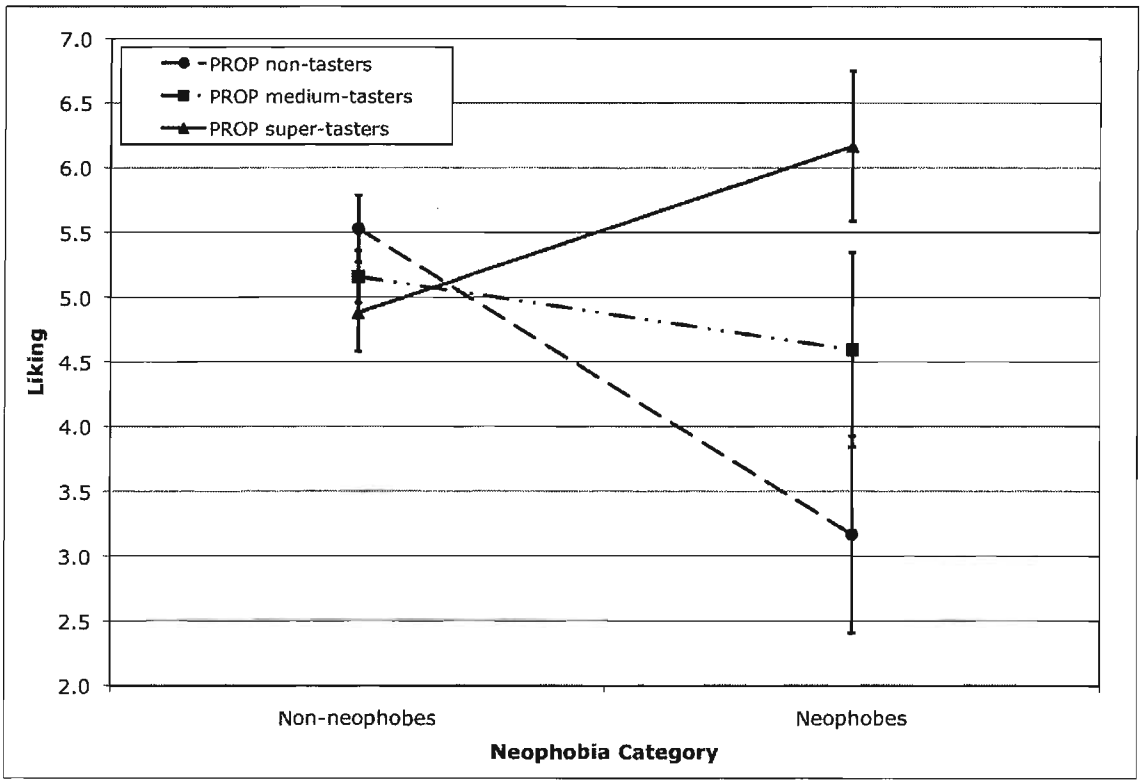
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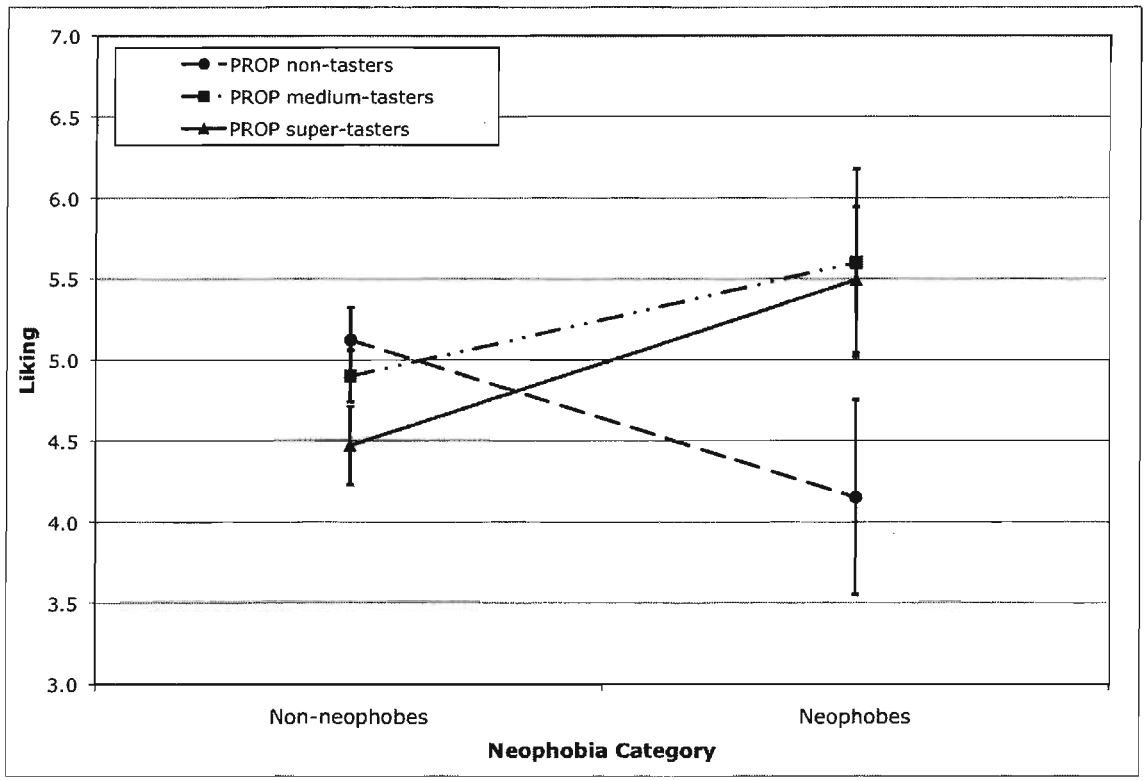
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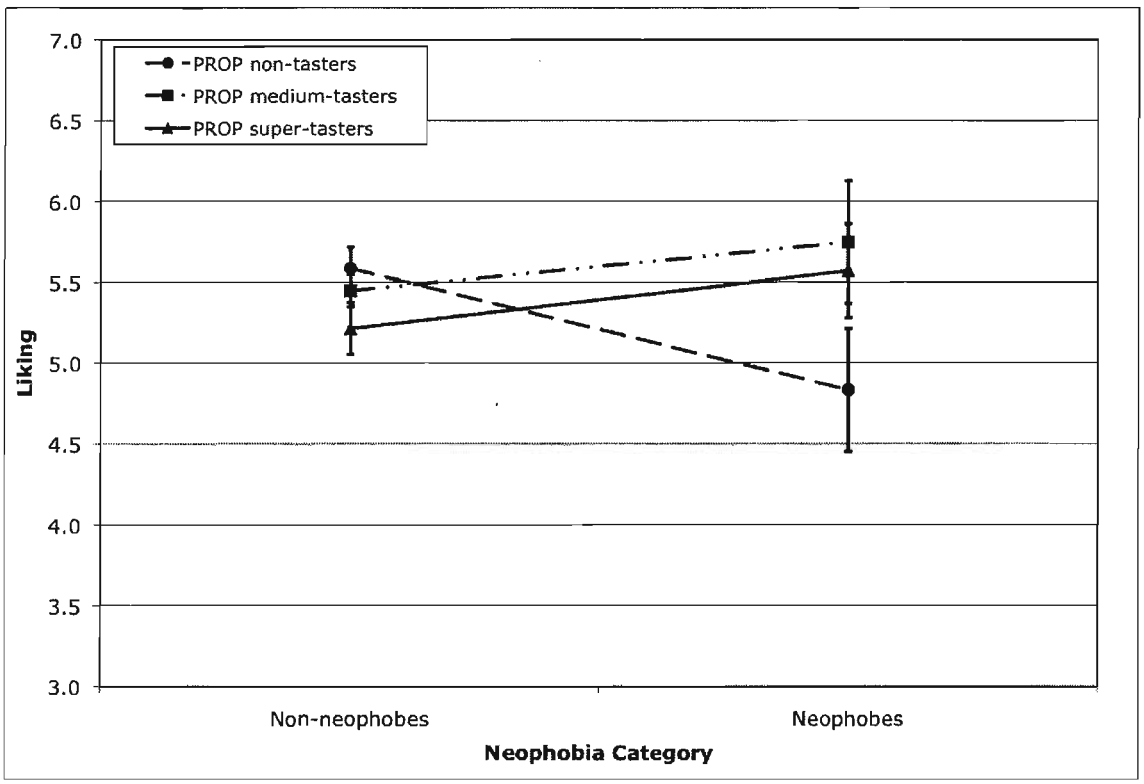
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Neophobia Category

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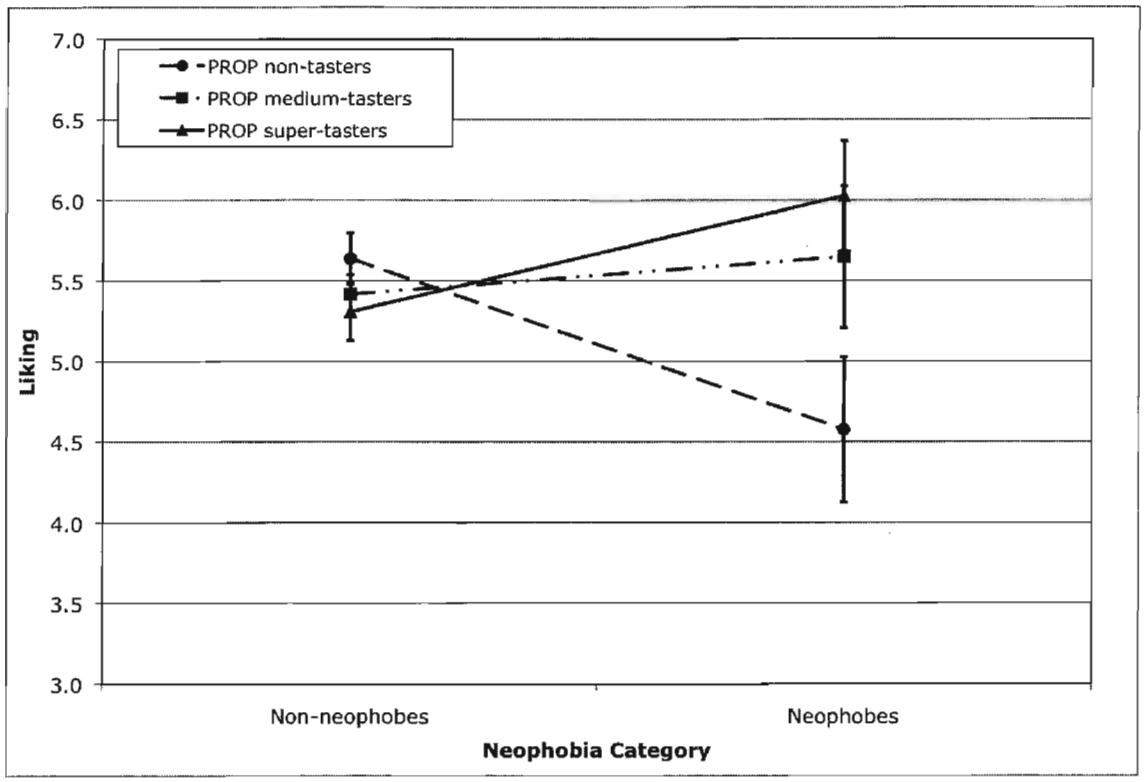


E)

Figure 4. PROP taster status (PTS)*neophobia interactions for the A) Dairy Food Group and B) Yogurt and C) Milk sub-groups, and the D) Fatty and E) Sweet Orosensory Groups. Line symbols represent mean liking ratings ±SE mean.

liking of the *Fruit* ($F(1,75)=3.85$, $p=0.05$), Seafood ($F(1,70)=3.48$, $p=0.06$), Pasta ($F(1,75)=3.08$, $p=0.08$) and Cooked Vegetable Food Groups ($F(1,75)=3.05$, $p=0.08$), the Mushy Orosensory Group, ($F(1,75)=3.48$, $p=0.07$), and the Raw Fruit Correlation Group ($F(1,75)=2.8$, $p=0.10$) approached significance.

No differences were observed between TTs and TnTs for BMI (TT=25.3±0.9; TnT=26.0±0.8; $F(1,75)=0.92$, $p=0.34$) or waist circumference (TT=87.2±2.8; TnT=87.6±1.7; $F(1,75)=0.46$, $p=0.50$) using either three-way (TTS, gender, and neophobia) or one-way ANOVA. A single TTS*neophobia interaction was observed for the Pie Food Group ($F(1,75)=4.49$, $p<0.05$), with TT non-neophobes providing higher liking scores than TT neophobes. As there were only 5 TT neophobes, and no overall TTS*neophobia trend is evident, this result is difficult to interpret. TTs (27.1±2.9) and TnTs (23.6±1.6) did not differ in their neophobia scores ($F(1,75)=1.35$, $p=0.25$) using one-way ANOVA.

PTS & PROP

The current work suggests lower liking of some characteristically bitter food items and cruciferous vegetables by pSTs, but none were rated differently between the three groups at the $p<0.05$ level of significance using either three-way (Table 2) or one-way ANOVA, with the exception of raw ($F(2,23)=5.9$, $p<0.01$) and cooked ($F(2,28)=4.3$, $p=0.25$) kohlrabi, where pMTs and pSTs differed significantly in a one-way ANOVA. Examination of the association between PROP intensity and liking of the bitter foods noted in Table 2 using Pearson's r revealed significant negative relationships for raw Brussels sprouts, and raw and cooked kohlrabi (Table 3). Differences in liking of the *Egg* ($F(2,125)=2.69$, $p=0.07$) and Cookies Food Groups ($F(2,126)=2.90$, $p=0.06$), and the Mushy Orosensory Group ($F(2,125)=2.39$, $p=0.10$) approached significance, while pNTs gave higher ratings for the Cream ($F(2,119)=3.29$, $p<0.05$) and Pasta ($F(2,126)=3.78$, $p<0.05$) Food sub-groups, as well as the Bitter Correlation Group ($F(2,117)=3.89$, $p<0.05$), Non-cruciferous Vegetable ($F(2,125)=8.30$, $p<0.01$), and Fat Correlation Group ($F(2,126)=9.59$, $p<0.001$).

Significant PTS*gender interactions were observed for the *Dairy* ($F(2,125)=5.11$, $p<0.01$), Milk ($F(2,125)=3.12$, $p<0.05$), and Frozen/sweet dairy Food

Table 2. Three-way ANOVA (PROP tasters status (PROP), gender, neophobia) results for PTS and liking of bitter foods, bitter beverages and cruciferous vegetables. Means, standard errors (±), number of subjects (n), F-value (F), and P-value (P) are presented. C=cooked, R=raw.

Food/beverage	**PROP non-tasters (n)**	**PROP medium-tasters (n)**	**PROP super-tasters (n)**	**F**	**P**
Black coffee	3.3±0.37(37)	3.3±0.29(60)	3.2±0.46(29)	1.81	0.17
Espresso	4.4±0.36(35)	4.7±.23(49)	4.4±0.43(25)	0.28	0.76
Tonic water	2.3±0.38(30)	3.2±0.29(56)	2.9±0.34(27)	0.38	0.68
Bok choi – C	5.1±0.31(25)	5.7±0.24(40)	5.5±0.35(19)	0.75	0.47
Bok choi – R	4.4±0.42(17)	4.5±0.33(25)	4.8±0.36(15)	0.69	0.51
Broccoli – C	5.7±0.22(36)	5.9±0.17(61)	5.9±0.25(29)	0.03	0.97
Broccoli – R	5.5±0.26(36)	5.0±0.25(57)	5.5±0.32(26)	0.55	0.58
Brussels sprouts – C	4.7±0.35(35)	4.8±0.27(60)	3.8±0.50(24)	0.59	0.56
Brussels sprouts – R	3.9±0.38(22)	2.9±0.32(34)	2.5±0.46(21)	0.65	0.52
Cabbage – C	4.5±0.32(37)	5.0±0.23(60)	4.2±0.43(28)	2.22	0.11
Cabbage – R	5.0±0.31(37)	4.6±0.25(54)	4.6±0.41(27)	1.81	0.17
Cauliflower – C	5.4±0.25(37)	5.4±0.19(61)	5.6±0.30(28)	0.95	0.39
Cauliflower – R	5.4±0.27(37)	4.8±0.24(59)	5.3±0.37(26)	0.06	0.94
Collard greens – C	4.9±0.4(18)	5.2±0.25(30)	4.6±0.47(16)	0.63	0.54
Mustard greens – C	4.9±0.41(16)	5.1±0.40(19)	4.5±0.40(13)	0.30	0.74
Kale – C	4.7±0.48(17)	5.0±0.34(27)	4.5±0.46(17)	0.12	0.89
Kale – R	4.5±0.49(13)	3.8±0.40(19)	3.5±0.47(15)	0.43	0.65
Kohlrabi – C	5.1±0.35(10)	5.6±0.16(10)	3.9±0.66(9)	3.23	0.06
Kohlrabi – R	5.1±0.40(8)	5.4±0.32((8)	3.0±0.78(8)	3.38	0.06
Radish – C	3.9±0.43(19)	3.8±0.27(35)	2.9±0.58(13)	0.66	0.52

Radish – R	4.2±0.34(37)	4.1±0.25(55)	3.9±0.45(24)	0.20	0.82
Rutabaga – C	5.2±0.42(18)	4.4±0.33(32)	3.7±0.45(18)	1.88	0.16
Rutabaga – R	4.0±0.46(12)	3.1±0.34(24)	2.9±0.42(15)	1.55	0.23
Turnip – C	4.5±0.32(34)	4.5±0.27(51)	3.6±0.51(22)	2.52	0.09
Turnip – R	3.5±0.43(20)	3.3±0.29(41)	2.2±0.41(17)	2.33	0.11
Watercress – C	4.5±0.34(15)	4.2±0.29(31)	4.7±0.53(14)	0.77	0.26
Watercress – R	4.7±0.32(22)	4.4±0.28(34)	5.3±0.32(22)	0.17	0.84
Grapefruit – R	5.5±0.31(37)	5.3±0.23(59)	4.8±0.41(28)	0.91	0.41
Horseradish – hot	3.8±0.44(33)	4.6±0.30(54)	3.3±0.43(25)	0.39	0.68
Horseradish – medium	4.1±0.43(33)	4.6±0.29(54)	3.9±0.48(25)	0.01	0.99
Horseradish – mild	4.3±0.42(34)	4.4±0.26(55)	4.1±0.46(25)	0.37	0.69

Table 3. Correlations (Pearson's r) between perceived PROP intensity and liking of bitter foods, bitter beverages and cruciferous vegetables. Number of subjects (n) is presented. * and ** indicate P<0.05 and P<0.01, respectively. C=cooked, R=raw.

Food/beverage	**r-value**	**N**
Black coffee	-0.03	126
Espresso	-0.17	109
Tonic water	-0.06	113
Bok choi – C	0.09	84
Bok choi – R	0.13	57
Broccoli – C	0.06	126
Broccoli – R	0.02	119
Brussels sprouts - C	-0.11	119
Brussels sprouts - R	-0.26*	77
Cabbage – C	-0.04	125
Cabbage – R	0.03	118
Cauliflower – C	0.13	126
Cauliflower – R	0.07	122
Collard greens - C	-0.19	64
Mustard greens - C	-0.28	48
Kale – C	-0.15	61
Kale – R	-0.23	47
Kohlrabi – C	-0.46*	29
Kohlrabi – R	-0.68**	24
Radish – C	-0.20	67
Radish – R	-0.13	116
Rutabaga – C	-0.22	68
Rutabaga – R	-0.21	51
Turnip – C	-0.04	107
Turnip – R	-0.09	78
Watercress – C	0.12	60
Watercress – R	0.18	78

Grapefruit – R	-0.07	124
Horseradish - hot	-0.10	112
Horseradish – medium	-0.08	112
Horseradish - mild	-0.04	114

Groups ($F(2,125)=(3.22, p<0.05)$, with male pNTs providing lower liking ratings than female pNTs. Similar trends were observed for pNTs in their liking of the Yogurt and Cream Food Groups, as well as the *Grain* and *Fruit* Food Groups and their sub-groups, and the majority of sub-groups under the *Dessert* Food Group. PTS*gender interactions were also observed for Sweet and Fatty Orosensory Groups, and again, male pNTs provided lower liking ratings than female pNTs. The same trend was observed for pNTs for all Orosensory Groups. Additionally, a significant interaction was observed for the Fat Correlation Group ($F(2,125)=3.82$, $p<0.05$). PTS*gender interactions must be interpreted with caution, as there were only 8 pST males in the cohort.

Food Neophobia

The perceived intensity of PROP bitterness was not correlated with neophobia scores ($r=0.02$, $p=0.82$) and, using one-way ANOVA, neophobia did not differ by PTS group ($F(2,125)=1.09$, $p=0.34$; pNTs=24.1±2.1; pMTs=22.6±1.4; pSTs=26.7±2.5). Using two-way ANOVA, PTS*neophobia interactions were observed. pST neophobes reported higher liking of the *Dairy* ($F(2,125)=5.05$, $p<0.01$), Yogurt ($F(2,125)=4.9$, $p<0.01$) and Milk ($F(2,125)=5.39$, $p<0.01$) Food Groups than pST non-neophobes, while pNT neophobes rated liking higher than pNT non-neophobes. Similar trends were observed for pSTs and pNTs for the Cream and Frozen/sweet Dairy Food Groups. Additionally, similar trends were observed for pSTs for the *Grain* and *Dessert* Food Groups, with the exception of the Hot Cereal and Cake sub-groups, respectively. Interestingly, the opposite trend was observed for the *Fruit*, *Vegetable*, and *Meat* Food Groups, and all their sub-groups, with pST non-neophobes providing higher liking scores than pST neophobes. Significant PTS*neophobia interactions were also observed for the Sweet ($F(2,125)=3.71$ $p<0.05$) and Fatty ($F(2,125)=3.37$, $p<0.05$) Orosensory Groups, again with pST neophobes and pNT non-neophobes liking both groups more than pST non-neophobes and pNT neophobes, respectively. The same trend was observed for pNTs for all Orosensory Groups, with the exception of Salty/Savoury. The opposite trend of pST neophobes providing lower liking scores than pST non-neophobes was observed for the Hot, Mushy, Salty/Savoury, and Bitter Orosensory Groups. PTS*neophobia

interactions should also be interpreted with caution, as there were 7, 9, and 6 individuals that were categorized as pNT, pMT, and pST neophobes, respectively.

BMI and WC

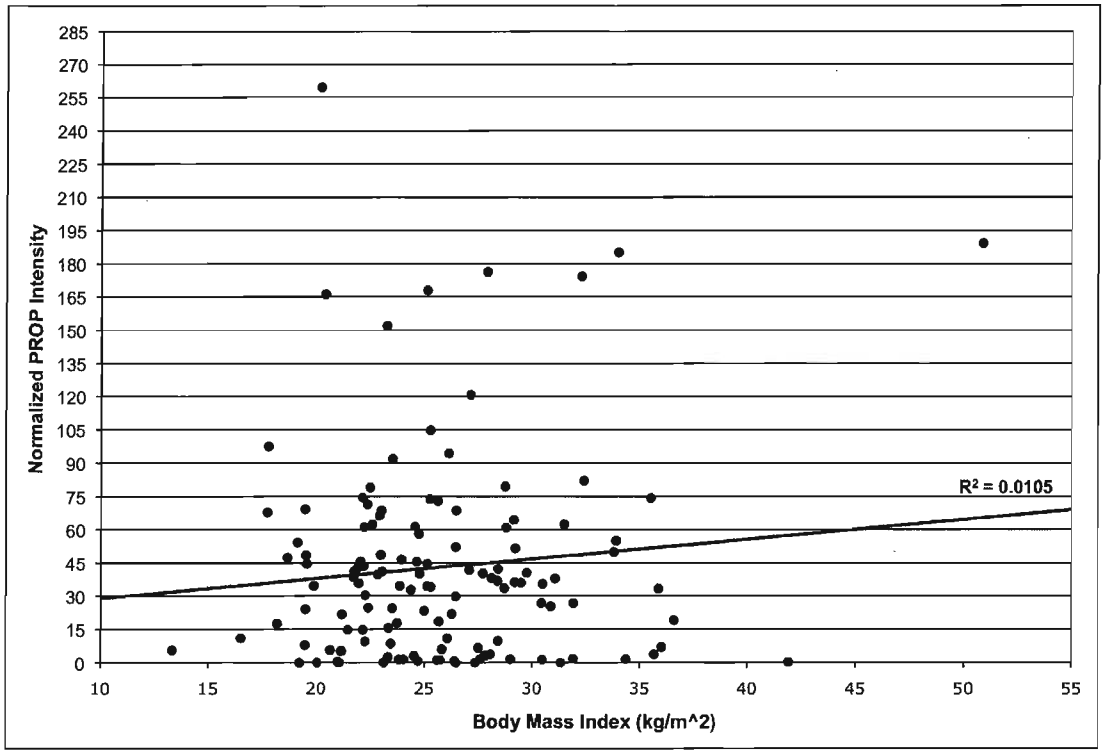
BMI ($F(2,125)=0.32$, $p=0.73$) and WC ($F(2,125)=0.33$, $p=0.72$) did not differ between pNTs (BMI=26.1±0.9; WC=89.1±2.0), pMTs (BMI=25.2±0.6; WC=86.7±1.8), and pSTs (BMI=26.2±1.2; WC=89.2±2.9) when analysed with three-way (PTS, neophobia, and gender) or one-way ANOVA (data not shown). The same null result was obtained when only the female cohort was analysed with both one-way and two-way (PTS and neophobia) ANOVA (data not shown). ANOVA revealed no significant differences between PTS groups for BMI ($F(2,82)=0.34$, $p=0.71$) and WC ($F(2,82)=1.1$, $p=0.33$). As with the whole cohort, female pMTs (BMI=24.3+/0.7; WC=81.7±1.6) had the lowest BMI and WC, while pNTs (BMI=26.4±1.2; WC=87.8±2.3) and pSTs (BMI=26.2±1.7; WC=86.5±3.6) were very similar in both measures. Not surprisingly given this result, the perceived intensity of PROP bitterness was not correlated with BMI ($r=0.10$, $p=0.26$) nor waist circumference ($r=0.09$, $p=0.34$), as shown in Figure 5. BMI and waist circumference were positively and significantly correlated with each other ($r=0.85$, $p<0.001$) and with age ($r=0.20$, $p<0.01$ and $r=0.30$, $p<0.01$, respectively).

Discussion

TTS

The primary objective of the current work was to investigate possible differences in food liking between TTs and TnTs. The differences between these two groups in their perception of orosensory stimuli may be genetic, and are comparable to those observed between PTS groups (Bajec et al. 2008a; Talavera et al. 2005). As such, we hypothesized that TTs would report greater dislike of food items with strong tastes, textures, or flavours than TnTs due to the intensity with which they perceive them. Additionally, the possible association of TTS with neophobia and its impact on BMI and WC was examined.

A cursory examination of the results suggests that texture may mediate differences between TTs and TnTs in food liking. For instance, TTs gave lower liking



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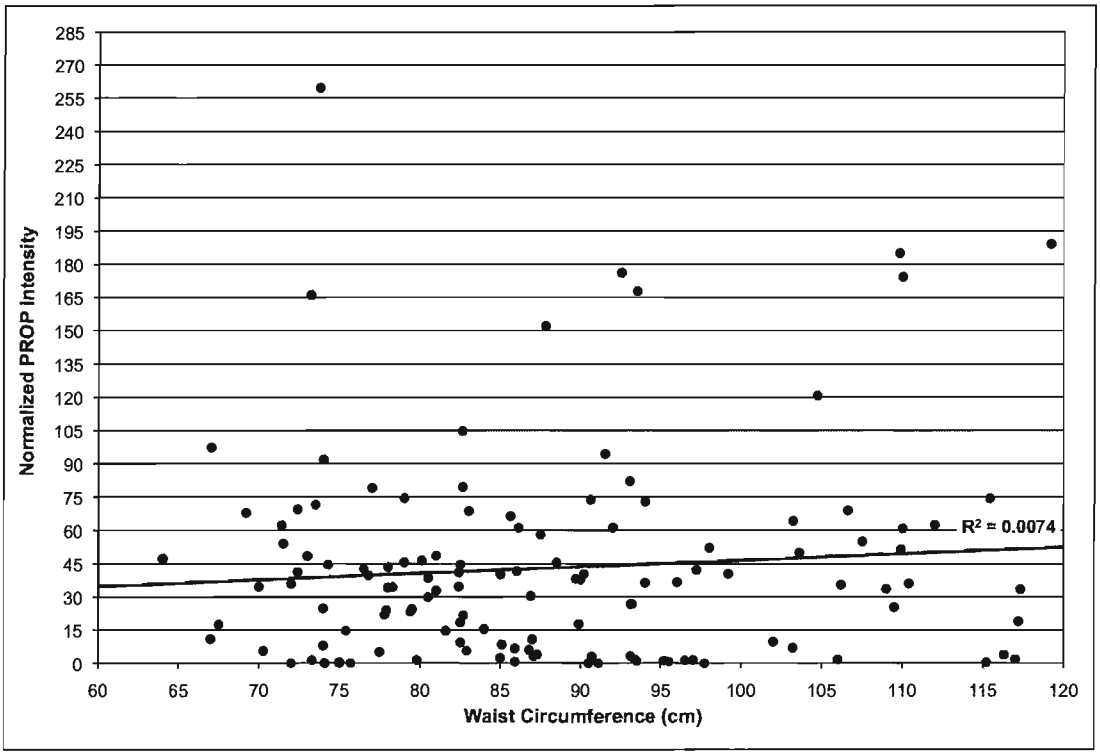

B)

Figure 5. Scatterplots with regression lines for perceived PROP intensity and A) BMI, and B) waist circumference.

scores for cooked fruits and vegetables and the Mushy Orosensory Group (composed of mainly cooked or preserved items, typically softer than their raw form), suggesting they like soft foods less than TnTs. FP density, which has been used as a measure of oral tactile sensitivity (Bartoshuk, 2000), does not differ between TTs and TnTs (Bajec et al. 2008a). However, as other areas of the oral cavity are also populated with mechanoreceptors (reviewed in: Bajec & Pickering, 2008b), tactile sensitivity between the two groups may differ independently of FP density. TTs perceive the intensity of astringency from alum with greater intensity than TnTs, and they report lower liking ratings for dry red wine (Bajec & Pickering, 2009).

Interestingly, Bajec et al. (2008a) did not find significant differences between TTS groups in their perception of PROP intensity, while here TnTs provided higher liking scores than TTs for the Bitter Correlation Group, which is composed mainly of cruciferous vegetables containing the thiourea moiety common to PTC and PROP. Green et al. (2004a), however, did find that TTs rated PROP higher than TnTs. The discrepancy in results may be due to the different PROP concentrations used by the two groups.

TTS was not associated with BMI or waist circumference, suggesting that TTS, unlike previous reports for PTS (Goldstein et al. 2005), is not linked with weight-related anthropometric measures. However, the lower liking scores of TTs for *Fruit*, Cooked Fruit and Cooked Vegetable Food Groups, and the Cooked Fruit/Vegetable Correlation Group may have health implications. The phenomenon of thermal taste is thought to work through the TRPM5 channel as opposed to a specific receptor, which differs greatly from the receptor mechanism of PROP perception (Duffy et al. 2004a; Talavera et al. 2005). Additionally, the advantage of TTs over TnTs in their perception of orosensory stimuli is hypothesized to work through a central nervous system gain mechanism (Green et al. 2005), which may influence diet and health through more complex multi-factor cognitive interactions.

Thermal taste does not manifest in all TTs in the same way; some TTs perceive sweetness on heating the tongue, while others perceive bitterness on cooling the tongue, and still others perceive saltiness (Cruz et al. 2000). Further characterization of the different thermal taster 'subtypes' may provide better insight into their psychophysical

characteristics and food preferences, but would require a larger number of subjects than surveyed here. Forty percent of the subjects evaluated in this study could not be categorized as either TTs or TnTs due to either the inconsistency of their responses or their response of a non-taste oral sensation. Further examination of this group of individuals and the basis for their reports would further elucidate the thermal taste phenomenon. Additionally, examination of the association between TTS and liking of sampled foods is warranted to further assess whether texture is a driver of liking for TTs.

PTS & PROP

The perceived intensity of PROP bitterness is a marker for individual variation in oral sensation that is hypothesized to affect food liking and consumption through its influence on perceived intensity of tastes (reviewed in Tepper, 2008; Duffy, 2007). Whether or not PTS actually affects food preference remains controversial (Drewnowski, Henderson & Cockroft, 2007). PTS has been suggested to influence vegetable preference and intake through its association with the perceived intensity of vegetable bitterness (Dinehart et al. 2006). Indeed, PAV/PAV individuals perceive the bitterness from cruciferous vegetables, which contain glucosinolates - a class of anti-thyroid compounds that include the thiourea moiety (N-C=S) common to PROP and PTC - more intensely than those with the AVI/AVI genotype (Sandell et al. 2006). A number of studies have reported lower preference for cruciferous vegetables and other bitter foods and beverages, including bitter green vegetables, coffee, grapefruit juice, tonic water, horseradish and cabbage (Drewnowski et al. 1999a; Dinehart et al. 2006; Lanier et al. 2005; Drewnowski et al. 2000; Drewnowski et al. 1997; Drewnowski et al 1998; Duffy et al. 1999; Villarino et al. 2009; Drewnowski, Henderson, Levine & Hann, 1999c). The current work suggests an inverse relationship between PROP intensity and liking for some of these food items. PTS groups differed in their liking ratings of the Bitter Correlation Group, a group composed of a subset of the food and beverage items examined independently whose liking scores negatively associate with perceived PROP bitterness intensity, but not for the Bitter Orosensory Group. These results suggest that the relationship between PROP intensity and bitter food liking is complex, and

dependent on more than just the perceived bitterness intensity of the food, as many of the foods in the Bitter Orosensory Group or the predominant bitter compound in the food item have previously been reported as differing in intensity between the PTS groups.

pNTs were expected to like fatty and creamy foods more than pSTs (Tepper et al., 2004). The near significance of pNTs' higher liking of eggs and soft or 'Mushy' foods suggests that the differences between the groups in their perception of texture may extend beyond fatty and creamy foods. FP are surrounded and sometimes inhabited by trigeminal innervation, which transmits somatosensory information from mechanoreceptors that respond to mechanical stimuli including friction, thickness, and viscosity (reviewed in: Bajec et al. 2008b). pSTs, who have a significantly higher FP density than either of the other groups (Bajec et al. 2008a; Bartoshuk et al. 1994; Tepper et al. 1997; Reedy et al. 2009), are hypothesized to dislike creamy and fatty foods due to their increased responsiveness to the tactile sensations they produce (Tepper, 2004). The lower liking of the Mushy Orosensory Group by pSTs may be due to the same mechanism, which is likely also responsible for the differences in liking scores for the Cream Food Group.

It is interesting to note that female pNTs appear to like sweet and fatty foods less than male pNTs, and that male PTS groups were more clearly separated in their liking of the types of foods than their female counterparts. While other studies have demonstrated relationships between gender and food liking, particularly for sweet, fat, and creamy foods, those relationships were between PTS groups and within genders (Duffy et al. 1999; Tepper et al. 1998; Keller et al. 2009; Keller & Tepper, 2004), unlike those observed here.

Food Neophobia

Food neophobia, the fear of trying new foods leading to food avoidance (Pliner et al. 1992), has been shown to moderate the effect of PTS on food liking in some studies (Ullrich et al. 2004). Using food adventurousness, defined as the frequency of trying new foods, Ulrich et al. (2004) found that food adventurous PROP tasters liked more foods than their non-adventurous counterparts, while adventurousness had little

influence on liking behaviour in non-tasters. The findings presented here suggest a more complex relationship between PTS and neophobia, and is worth examining further. Additionally, if liking functions as a proxy for consumption, as recent evidence suggests (Duffy et al. 2009; Duffy, Lanier, Hutchins, Pescatello, Johnson & Bartoshuk, 2007), a detailed examination of the consumptive behaviours of pST neophobes may provide insight into the conflicting reports regarding the relationship between PTS and health (Duffy, 2007; Tepper, 2008; Drewnowski et al. 2007).

BMI and WC

We found no association between PROP and BMI or WC. The relationship between PROP and BMI remains controversial (Tepper, 2008; Drewnowski et al. 2007). The current work is limited in that dietary restraint, which may moderate the relationship between PROP and BMI (Tepper et al. 2002), was not taken into account. This study does, however, add to a growing body of data that suggests that PROP responsiveness does not associate with BMI and/or waist circumference (Dinehart et al. 2006; Duffy et al. 2000; Drewnowski et al. 2007; Drewnowski, Ahlstrom-Henderson & Barratt-Fornell, 1998b; Yackinous & Guinard, 2001), even when dietary restraint is considered (Hayes et al. 2007).

Conclusions

The current work suggests some association between TTS, PTS and food preferences, which in the case of TTS may be driven by texture. Previously reported PTS associations with BMI and WC were not supported here, and these results do not show a relationship between TTS and BMI, or TTS and WC. Additionally, PTS and TTS do not appear to associate with neophobia, although some PTS*neophobia interactions were observed. The differences in food liking associated with PTS and TTS described here support previous findings indicating that these phenotypes function independently with respect to orosensory perception (Bajec et al. 2008a). The interactions of PTS with gender and neophobia suggest that these variables should be further examined in relation to food liking. Determining the influence of TTS on sampled food and beverages and obtaining descriptive measures of the orosensory experiences of TTs and

TnTs may provide better insight into their perception of and preference for food and beverages.

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CHAPTER 7: SUMMARY, GENERAL DISCUSSION AND FUTURE RESEARCH

Summary

Due to its positive relationship with the perceived intensity of orosensory stimuli, its genetic basis (Kim et al., 2003; Bufe et al., 2005; Duffy et al., 2004; Hayes et al., 2008), and its association with food preference and health and disease states (reviewed in: Tepper et al. 2009; Duffy, 2007; Tepper, 2008), PROP responsiveness has long been used as an index of individual variation in oral sensation. Recently, other indices of individual variation have been described, including TTS (Cruz & Green, 2000). While it is known that TTS associates with super-tasting (Green & George, 2004; Green et al., 2005), this relationship has not been thoroughly described. This dissertation examined the influence of biological sources of individual variation, including PTS, TTS, gender, FP density, and age on the perception of orosensory stimuli, including the sensation of astringency. PTS and TTS were both included here to determine if a relationship exists between them and to compare these two proposed indices of individual variation in oral sensation. Further, the influence of sources of individual variation and the perception of oral sensations on food and alcohol behaviours was studied to examine the influence of taster non-taster status on real-life hedonic and consumptive measures.

The associations of PTS and TTS with the perceived intensity of sweet, sour, salty, bitter, two concentrations of metallic and astringent stimuli were examined. Interactions between PTS and TTS were investigated, and FP density and SFR were determined. Both PTS and TTS were associated with perceived intensities of orosensory stimuli. pSTs rated all oral stimuli as more intense than pNTs. TTs gave higher logged intensity ratings than TnTs for all oral sensations including temperature, with the exception of metallic flavour, from a low concentration of iron sulfate, and PROP. Examination of Eta-squared values showed that PTS had a greater effect on perceived intensities than did TTS for most sensations. No PTS*TTS interaction was found for any oral stimuli. In contrast with PTS, TTS and the perceived intensity of the orosensory stimuli examined were not associated with FP density. SFR was not

associated with PTS, TTS, or any of the orosensory stimuli examined, including astringency.

TTS and PTS effects on the perception of orosensory stimuli at two temperatures (5°C and 35°C) using TI methodology were examined. Two hypotheses were put forth in this study: i) that if TTs are more responsive to both chemical and thermal stimuli, they would perceive stimuli delivered at different temperatures differently from TnTs, and ii) that if the channels involved in the transduction of taste are taste/temperature coincidence detectors and are more sensitive to temperature in TTs, then TTs may respond differently to the chemical that elicits the taste they perceive upon thermal stimulation (i.e., sweetness on warming, bitter on cooling, etc.) at different temperatures. A trend of TTs reporting higher maximum intensities was observed, which held regardless of temperature. Although some differences between PTS groups was observed, PROP bitterness was not associated with the maximum perceived intensity of the orosensory stimuli examined and the anticipated PTS effect on this parameter was not observed. No PTS*TTS interaction was observed for any TI parameter, and Eta squared values indicate PTS exerts a greater influence on TI parameters than TTS. Additionally, the results presented here suggest that temperature influences the maximum perceived intensity of astringency, bitterness, sourness, but not sweetness.

It was hypothesized that PTS, TTS, and gender would affect surveyed liking and intake of alcoholic beverages. Liking of 43 alcoholic beverages was indicated using a 7-point Likert scale, and alcoholic beverage consumption frequency and quantity were measured. While all three sources of individual variation were found to affect liking and consumption of some alcoholic beverages, gender affected the greatest number of parameters and beverages, with females generally liking and consuming alcoholic beverages less than males. TTs and pMTs liked some individual beverages and beverage groups more than their non-taster counterparts, and some differences in consumption were observed between the taster groups. Partitioning subjects into PTS and TTS taster groups then examining the associations between perceived intensity of orosensory stimuli and liking and consumption of alcoholic beverages suggests that liking is driven by different associations between the different groups. Eta-squared

values suggest that PTS had the greatest effect on liking, while multiple regression indicated that age and gender were the best predictors of alcoholic beverage liking and consumption. Significant associations were observed between liking of beverage types and their consumption.

The association of TTS and PROP responsiveness with food liking, BMI, WC, and neophobia were examined. Subjects rated liking of 332 food and beverage items, which included different preparations of foods, using a 7-point hedonic scale. TTS did not associate with BMI or WC, and contrary to previous studies, neither did PROP responsiveness. TnTs' greater liking of cooked fruits and vegetables over TTs suggests differences between TTS groups may be texturally driven. As expected, liking of bitter and fatty foods and cream was inversely related to PROP responsiveness.

General Discussion and Future Research

Both indices of individual variation in oral sensation were associated with responsiveness to orosensory stimuli. Categorization as a taster for either of the two indices examined here was positively associated with the perceived intensity of orosensory stimuli, including two concentrations of alum, as TTs and pSTs provided higher ratings than TnTs and pNTs, respectively. Previous work suggested that TTs were super-tasters for all orosensory stimuli tested, with the exception of chemesthetic stimuli (Green et al., 2005). Here, alum was rated higher by TTs, indicating that the perceived intensities of chemesthetic and tactile stimuli cannot act as predictors for each other, as was expected given the different mechanisms involved in their transduction. The association of PROP responsiveness, but not FP density with the perceived intensity of the astringent solutions suggests that the astringency elicited by alum is dependent on more than innervation density. Unexpectedly, in the log-treated data TTs did not report PROP intensity as significantly higher than TnTs. PROP concentration and/or stimulus delivery method may be a factor in the discrepancy between the result reported here and that of others (Green & George, 2004), however, the lack of TTS effect on PROP bitterness intensity ratings, and the lack of interaction between TTS and PTS effects on any orosensory stimuli examined in both of the psychophysical studies presented here strongly suggests the two indices function independently.

Interestingly, the pST advantage observed for perceived intensity of orosensory stimuli at ambient temperature was not observed using cool or warm stimuli. Indeed, the TI curves for pSTs and pNTs often reached the same maximum intensity, and for sucrose at both temperatures and warm quinine pNTs reported higher maximum intensity ratings. Sweetness intensity from sucrose has been associated with PROP responsiveness in numerous studies (Gent & Bartoshuk, 1993; Lucchina et al., 1998; Hayes & Duffy, 2007), including Chapter 3, which makes this result particularly surprising. Whether static intensity measures yield the same results at the two temperatures, or if this is a result of the use of TI methodology should be examined. To the author's knowledge, there are no studies in the literature describing the association or lack of association between PROP responsiveness and the intensity of orosensory stimuli using TI methodology. If results similar to those reported here are found, the relationship between PROP and the perception of orosensory stimuli should be reevaluated. It is possible that psychological differences between PTS groups (Macht & Mueller, 2007) extend to the cognitive task of rating the intensity of orosensory stimuli using static methods (e.g., the task of making a static rating leads them to inflate their scores) that are not elicited when intensity ratings must be made in a method that forces the participant to concentrate on the perception over time. A TI study of PTS effects on orosensory stimuli at room temperature would help determine whether rating methodology influences PTS groups differently.

The postulated hypothesis that if the channels involved in the transduction of taste are taste/temperature coincidence detectors and are more sensitive to temperature in TTs, then the TI response of TTs to the tastant that chemically elicits the taste they perceive upon thermal stimulation (i.e., sweetness on warming, bitter on cooling, etc.) may differ at different temperatures was not supported; however, a larger study of TT sub-groups is required to draw a meaningful conclusion as the numbers of each TT sub-group in this study were somewhat low. The detailed examination of TT sub-groups would also allow determination of whether the thermal taste perceived influences the individual's liking for that taste sensation and foods that exhibit it. While there was a trend for TTs to produce higher maximum intensities than TnTs for most orosensory stimuli, it is unclear what this finding means. Green and colleagues (Green & George,

2004; Green et al., 2005) have suggested that super-tasting results from a central nervous system gain mechanism in the afferent system mediating flavour perception. The globality of the trend for TTs to rate the perceived intensity of all orosensory stimuli presented, including the perceived intensity of thermal and tactile stimuli, which are mediated by the somatosensory system, suggest that this might be true for TTs. It would be of interest to determine whether TTs are more responsive to stimuli presented to other senses (e.g., vision, audition) as well as the gustatory, olfactory, and some modalities of the somatosensory system. TTs do note perceive chemesthetic stimuli more intensely than TnTs, suggesting that the TT advantage does not extend to nociceptive oral stimuli (Green et al., 2005). Whether TTs are more responsive to emotional stimuli than TnTs might shed light on their cognitive processes and whether general heightened responsiveness is a personality trait of the TT group. Greater emotional reactivity to negatively charged stimuli has been observed in the responses of pSTs compared to those of pNTs (Macht & Mueller, 2007). Interestingly, however, if the generality of heightened response were a defining feature of a central nervous gain mechanism (Green & George, 2004; Green et al., 2005), it would seem that the association between PROP responsiveness and orosensory stimuli is not centrally-mediated, at least not exclusively. If it were, one would expect orosensory stimuli at all temperatures to be associated with PROP responsiveness or PTS, which was not observed here.

Although PTS, TTS and gender were found to affect liking and consumption of some alcoholic beverages, gender affected the greatest number of consumption parameters and beverages, with females generally liking and consuming alcoholic beverages less than males. The trend of pMTs liking alcoholic beverages more than pSTs or pNTs prompted the 'best of both worlds' hypothesis, which states that if pMTs' responsiveness falls between that of pNTs and pSTs, then pMTs may have an advantage where the perceived intensity of the sensory experience allows them to enjoy the complexity of alcoholic beverages without being overpowered by the bitterness found in many alcoholic beverages and the heat from ethanol. While other studies have found pMTs' orosensory responsiveness to be between that of pSTs and pNTs, here pMTs' characteristic seem closer to pNTs. Their perception of the intensity of orosensory

stimuli at ambient temperature did not differ significantly from pNTs. It is also interesting to note that using TI methodology at two temperatures, pMTs rated all stimuli lower in maximum intensity than pSTs or pNTs. Given that this group comprises the greatest proportion of the population, and thus consumers, the liking and responsiveness of these medium-tasting individuals warrants further investigation.

A trend for TnTs to like all alcoholic beverage types more than TTs was observed. Based on the protection hypothesis put forth for pSTs, this finding was expected given TnTs' lower responsiveness to prototypical tastants, flavours, and astringent stimuli. The individual beverages that TnTs rated higher than TTs tended to be high alcohol (e.g, bourbon), and astringent (i.e., dry red wine) in nature. Although differences in the perceived intensity of ethanol between the thermal taster groups have not been examined, TTs' higher responsiveness to thermal heat on the tongue, as reported here and by Green and George (2004), may be related to their greater dislike of high alcohol beverages. TnTs' perceived temperature intensity associated with the liking of some mixed spirits, which was not observed for TTs, suggesting that temperature may play a role in TTs' lower liking of some beverages. TTS was not a predictor of alcoholic beverage liking in multiple regression analysis. Interestingly, however, the perceived intensity of prototypical tastants, astringency, metallic flavour and temperature were correlated with liking for a greater number of alcoholic beverages in TnTs, suggesting that orosensory perception may have more influence on TnTs' liking of alcoholic beverages than for TTs.

Previously reported PTS associations with BMI and WC were not supported here, and these results do not show a relationship between TTS and BMI, or TTS and WC. However, TTs' lower liking scores for food groups comprised of fruits and vegetables may have health implications. TTs also gave lower liking scores for foods that were categorized as 'Mushy', suggesting they like soft foods less than TnTs. This finding, coupled with the alcohol results described above, suggests that TTs and TnTs differences in food and alcohol are texturally driven. A group of predominantly astringent foods affected by PTS or TTS was not found using the methods of analysis used here. Astringency is often difficult to separate from bitterness and sourness in simple solutions and complex food matrices alike (Lea & Arnold, 1978; Ishikawa &

Noble, 1995; Peleg et al., 1999), as such, future work should examine sampled foods specifically selected for having astringency as a dominant and easily recognizable attribute. The differences in food liking associated with PTS and TTS support the psychophysical finding that these phenotypes function independently. The interactions of PTS with gender and neophobia suggest that these variables should be further examined in relation to food liking, as their relationship appears complex and food-dependent. Determining the influence of PTS and TTS on sampled food and beverages and obtaining descriptive measures of the orosensory experiences of taster and non-taster groups may provide better insight into their perception of and preference for food and beverages.

The psychophysical data presented here suggest that, although a larger effect was observed using PTS, TTS may serve as a better proxy for the perceived intensity of orosensory stimuli, including astringency, as TTs were more consistent than pSTs in their presentation of super-taster characteristics. A drawback of using TTS as an indicator of individual variation is that the assessment protocol is time intensive and requires delicate instrumentation. The influence of indices of variation in oral sensation on the liking and consumption of alcohol and the liking of food is complex, and requires further research.

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APPENDIX A: SPEARMAN'S ρ VALUES FOR PROP RESPONSIVENESS AND TIME-INTENSITY PARAMETERS (CHAPTER 4)

Table A1. Spearman's ρ values and accompanying p-values for the correlation calculated between PROP responsiveness and TI parameters (as discussed in Chapter 4). * indicates a correlation with $p<0.05$.

Time-intensity parameter	Spearman's ρ	p-value
Cold Alum TM	-0.07	0.68
Cold Alum IM	0.23	0.13
Cold Alum D	0.09	0.55
Cold Alum AUC	0.07	0.65
Cold Alum IAng	0.13	0.41
Cold Alum IArea	-0.02	0.88
Cold Alum DAng	-0.10	0.55
Cold Alum DArea	0.08	0.61
Cold Alum IDelay	-0.24	0.11
Cold Alum IInt	0.05	0.74
Cold Quinine TM	-0.01	0.93
Cold Quinine IM	0.17	0.26
Cold Quinine Dur	-0.20	0.19
Cold Quinine AUC	-0.03	0.85
Cold Quinine IAng	0.11	0.47
Cold Quinine IArea	0.15	0.33
Cold Quinine DAng	0.21	0.18
Cold Quinine DArea	-0.11	0.47
Cold Quinine IDelay	-0.10	0.53
Cold Quinine IInt	0.15	0.34
Cold Citric Acid TM	-0.10	0.50
Cold Citric Acid IM	0.03	0.83
Cold Citric Acid Dur	-0.04	0.77
Cold Citric Acid AUC	-0.01	0.94
Cold Citric Acid IAng	0.09	0.55
Cold Citric Acid IArea	0.04	0.80
Cold Citric Acid DAng	-0.03	0.84
Cold Citric Acid DArea	-0.03	0.86
Cold Citric Acid IDelay	-0.23	0.14
Cold Citric Acid IInt	0.14	0.38
Cold Sucrose TM	-0.30	0.05

Cold Sucrose IM	-0.03	0.83
Cold Sucrose Dur	-0.39*	0.01
Cold Sucrose AUC	-0.11	0.48
Cold Sucrose IAng	0.25	0.11
Cold Sucrose IArea	-0.10	0.51
Cold Sucrose DAng	0.26	0.09
Cold Sucrose DArea	-0.15	0.33
Cold Sucrose IDelay	-0.11	0.49
Cold Sucrose IInt	0.06	0.72
Warm Alum TM	0.05	0.75
Warm Alum IM	0.06	0.68
Warm Alum Dur	-0.09	0.55
Warm Alum AUC	0.01	0.94
Warm Alum IAng	0.09	0.55
Warm Alum IArea	0.02	0.88
Warm Alum DAng	0.05	0.73
Warm Alum DArea	-0.02	0.92
Warm Alum IDelay	-0.19	0.22
Warm Alum IInt	-0.01	0.92
Warm Quinine TM	-0.29	0.05
Warm Quinine IM	0.00	0.98
Warm Quinine Dur	-0.27	0.08
Warm Quinine AUC	-0.16	0.29
Warm Quinine IAng	0.25	0.10
Warm Quinine IArea	-0.23	0.13
Warm Quinine DAng	0.21	0.17
Warm Quinine DArea	-0.13	0.41
Warm Quinine IDelay	-0.18	0.24
Warm Quinine IInt	-0.08	0.61
Warm Citric Acid TM	-0.13	0.41
Warm Citric Acid IM	0.10	0.50
Warm Citric Acid Dur	0.02	0.87
Warm Citric Acid AUC	0.07	0.65
Warm Citric Acid IAng	0.15	0.32
Warm Citric Acid IArea	-0.04	0.79
Warm Citric Acid DAng	-0.10	0.53
Warm Citric Acid DArea	0.08	0.58
Warm Citric Acid IDelay	-0.04	0.77
Warm Citric Acid IInt	0.14	0.37
Warm Sucrose TM	-0.27	0.08
Warm Sucrose IM	-0.11	0.47

Warm Sucrose Dur	-0.30	0.05
Warm Sucrose AUC	-0.16	0.30
Warm Sucrose IAng	0.07	0.66
Warm Sucrose IArea	-0.09	0.59
Warm Sucrose DAng	0.18	0.24
Warm Sucrose DArea	-0.16	0.32
Warm Sucrose IDelay	-0.09	0.58
Warm Sucrose IInt	-0.04	0.82

APPENDIX B: CONSENT FORM FOR CHAPTERS 3, 5, AND 6

INFORMATION STATEMENT AND CONSENT FORM

Name of Research Project: Psychological, demographic and behavioural variables underlying thermal and PROP taste status.

Department/Institute: Brock University; Department of Biological Science/ Cool Climate Oenology and Viticulture Institute

Principal Investigator: Ms. Martha Bajec, Ph.D. Student, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4719, mbajec@brocku.ca

Supervisor: Dr. Gary Pickering, Associate Professor, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4715, gpickeri@brocku.ca

Co-Investigator: Lynda VanZuiden, Research Assistant, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4719, lvanzuiden@brocku.ca

The Project:

The ability to taste the (potentially bitter and mildly unpleasant) compound 6-n-propylthiouracil (PROP), and the perceived intensity of this compound if detected, are largely genetically determined and may vary greatly from individual to individual. Sensitivity to PROP has been related to food preference and acceptance. Likewise, the ability to experience 'taste' sensations as a result of thermal stimulation has also been suggested to be genetically variant, and may have implications in food preference and acceptance.

The relationship between a person's PROP-taster status, thermal-taster status and various physiological and behavioural variables (i.e., taste bud numbers, salivary flow rate, physical dimensions, health, food-preference, food-adventurousness) has not been determined. This study will examine whether:

-there is a population/gender/age trend for thermal-taster status;
-an individual's PROP-taster status is correlated with their TTS;
-PROP-taster status or thermal-taster status are correlated with an individual's anthropometric measures and health?
-either PTS or TTS correlated with an individual's preferences for certain flavours/textures;
-flavour/texture preference correlated with food-adventurousness, or food exposure/cultural background?

The Procedure:

You are invited to participate in this study! 100-150 participants will fill-out questionnaires about health, demographics, food-preferences and food-adventurousness. They will also have their PROP taster-status and their thermal-taster status determined. PROP-taster status is determined by rating the intensity of a solution of PROP after an orally rinsing with it (and expectorating). Quickly cooling and heating a small area of

the tongue tip determines thermal-taster status. Papillae counts will also be taken, which are done by simply staining the tongue with blue food colouring and taking a few digital images. Saliva is stimulated by a citric acid rinse, following which saliva is collected for one minute to determine salivary flow rate. Other physiological measures, such as body dimensions (height, weight, waist circumference, hip circumference) will also be collected.
This information will also be used to recruit a panel for a future study regarding aroma/astringency interactions in wine and wine-related stimuli.

Benefits/Risks
The expected benefits for the participant and the scientific community are a greater understanding of the role of genetics and physiology in taste preference. The determination of thermal-taste status is very novel, as such, participants will be among a very few others (less than 300 people world-wide) who have had their thermal-taste status determined. In addition, participants will become more aware of their palates, and food preferences.
The expected risks are no great than those encountered in normal daily food and beverage consumption. While they may not be pleasant to everyone, all substances to be tasted are perfectly safe, and are of food-quality grade or better.
Participants will be entered into a draw for a $200 gift certificate for either the Brock University Book Store, or Chapter's (whichever the winner prefers; odds of winning are 1 in 150).

Voluntary Participation:
You are free to withdraw your participation in the research at any time, and if you do, any data collected from will immediately be destroyed.

Responsibilities:
The participant needs only to schedule an approximately 1.5 hour block of time to come to the Pickering lab (IH215) at Brock University. Times will be available during normal working hours, after working hours, and on weekends, as agreed upon by the participant and the Principle Investigator.

Publication of Results
It is expected that the results of this study will be published, in academic journals and presented at conferences. Please feel free to contact either the Principle Investigator, or the Supervisor at any time should any questions arise. Also, for more information on the progress or results of the study please contact either persons mentioned above, or consult the Pickering lab website at
http://www.brocku.ca/ccovi/pages/people/show.php?id=9.

Confidentiality
All data will be confidential, individual identities will not be disclosed to anyone outside of the researchers listed above. Paper data (i.e., physical measure tables, health and demographic questionnaires) collected during this study will be will be retained for 7 years. Digitally recorded questionnaires (i.e., those completed on a computer by the

participant, digital photographs) will be retained on compact disc for 7 years. Digital photographs will be retained indefinitely for training and presentation (if permitted by the participant, please see below). Data will be stored in a locked, private area accessible only to the Supervisor. Only the individuals listed above will have access to the data.

Ethics Clearance:

This study has been reviewed and received ethics clearance through the Research Ethics Board at Brock University (REB file# 05-258). If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at (905) 688-5550 Ext. 3035, reb@brocku.ca.

Consent:

The purpose of the research has been explained to me, including the potential risks/discomforts associated with the research. I have also been given the opportunity to ask questions about the research and received satisfactory answers, and know that I may continue to ask questions and receive satisfactory answers throughout the study.

I understand that I am free to withdraw my participation in the research at any time and that if I do I will not be subjected to any penalty or discriminatory treatment. I also understand my participation in this project is on a voluntary basis, and no remuneration will be provided by Brock University in exchange for my participation.

I understand that any information or personal details gathered in the course of this research about me are confidential and that neither my name nor any other identifying information will be used or published without my written permission.

This study has received this study has been received ethics clearance from the Brock University Research Ethics Board as per REB file# 05-258). I understand that if I have any complaints or concerns about this research I can contact:
Research Ethics Officer, Office of Research Services, Brock University, Ph: 905 688 5550, ext: 3035; **reb@brocku.ca**

Your Name:

.....

Signature:

.....

Date:.....

Please check this box if you ARE NOT interested in being contacted to participate in the aroma/astringency study, or any other study performed by the Pickering lab. ☐

Please check this box if you DO NOT wish to have your photograph (tongue only, no identification) used for publication (scientific journal or other publication) or training purposes. ☐

Please complete the following for entry in a draw (to be held at the completion of the study) to wine a $200 gift certificate for either Chapter's or the Brock University Bookstore (winner's choice).

Name:.....
Email address:.....
Phone number: .....

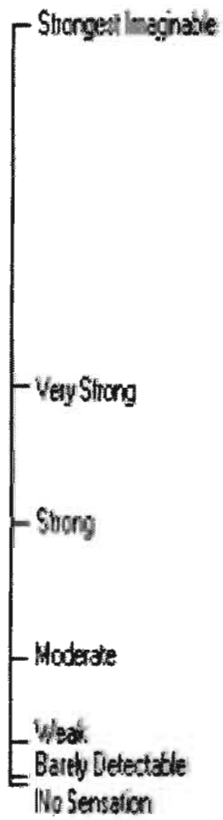
APPENDIX C: CHAPTER 3 SCALES

You are being asked to rate the intensity of a remembered sensation, namely, the **brightness of the sun when you are looking directly at it**, by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing. *Please mark the scale with a horizontal line only.*

brightness of the sun when you are looking directly at it



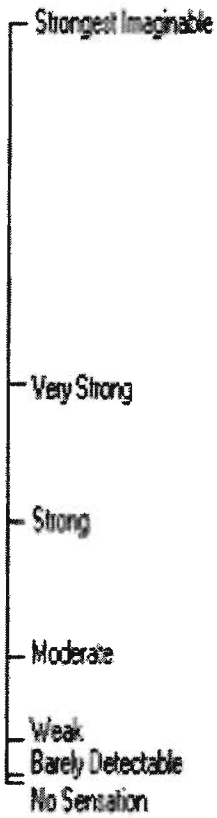
OTHER REMEMBERED SENSATIONS RATED

-coolness of an ice cold beverage
-burning sensation from eating a whole hot pepper
-pain from biting your tongue
-sourness of a lemon

You are being asked to rate the intensity of **sourness** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

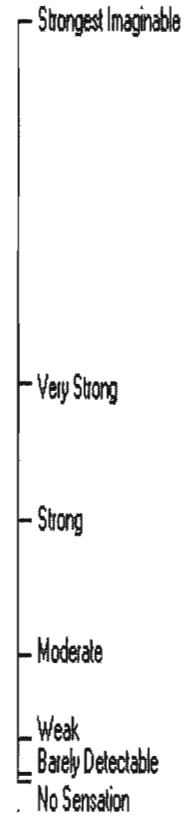
It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing.



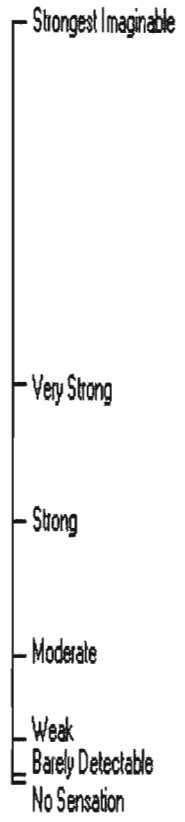
You are being asked to rate the intensity the sensations you experienced upon heating of your tongue by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

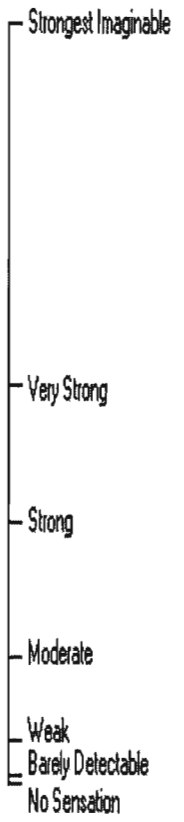
It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing.



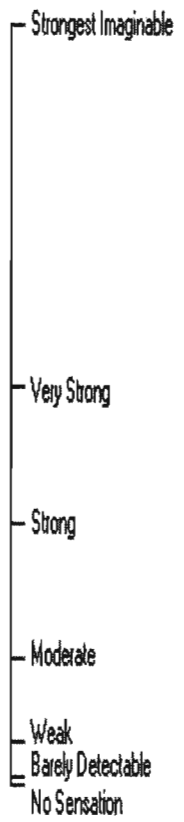
TEMPERATURE



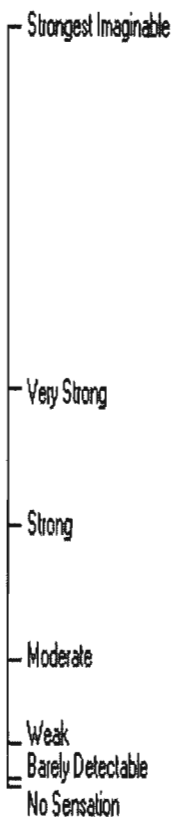
SWEET



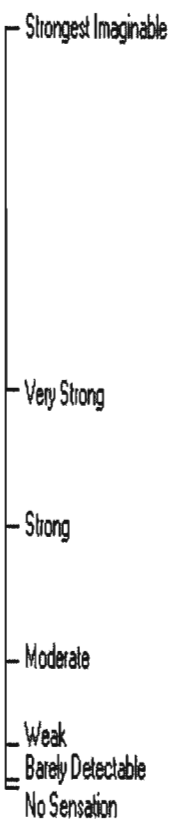
SALTY



SOUR



BITTER



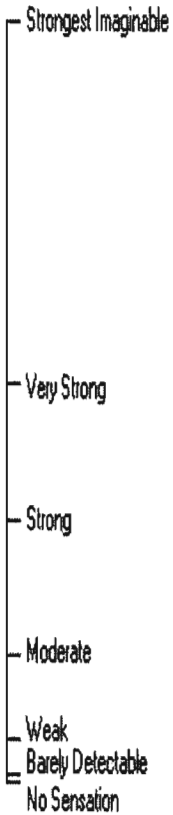
OTHER

SPECIFY:

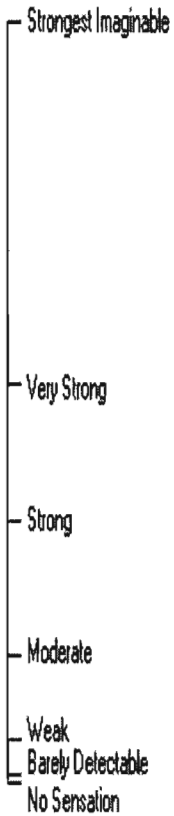
You are being asked to rate the intensity the sensations you experienced upon cooling of your tongue by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

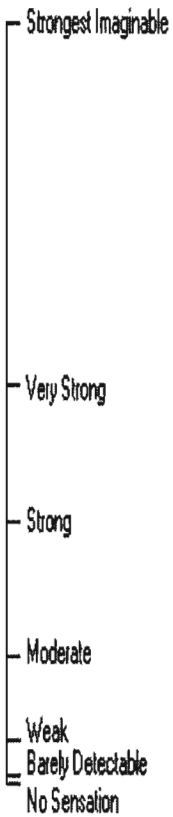
It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing.



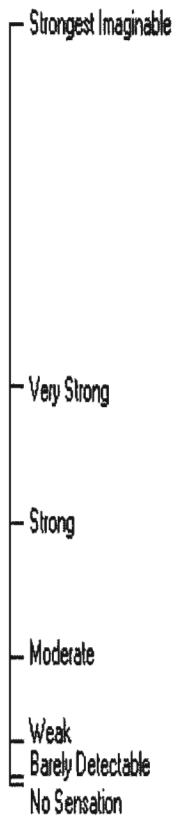
TEMPERATURE



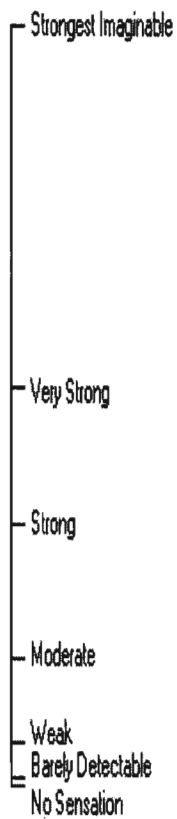
SWEET



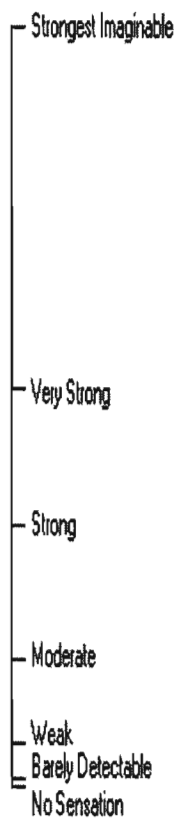
SALTY



SOUR



BITTER



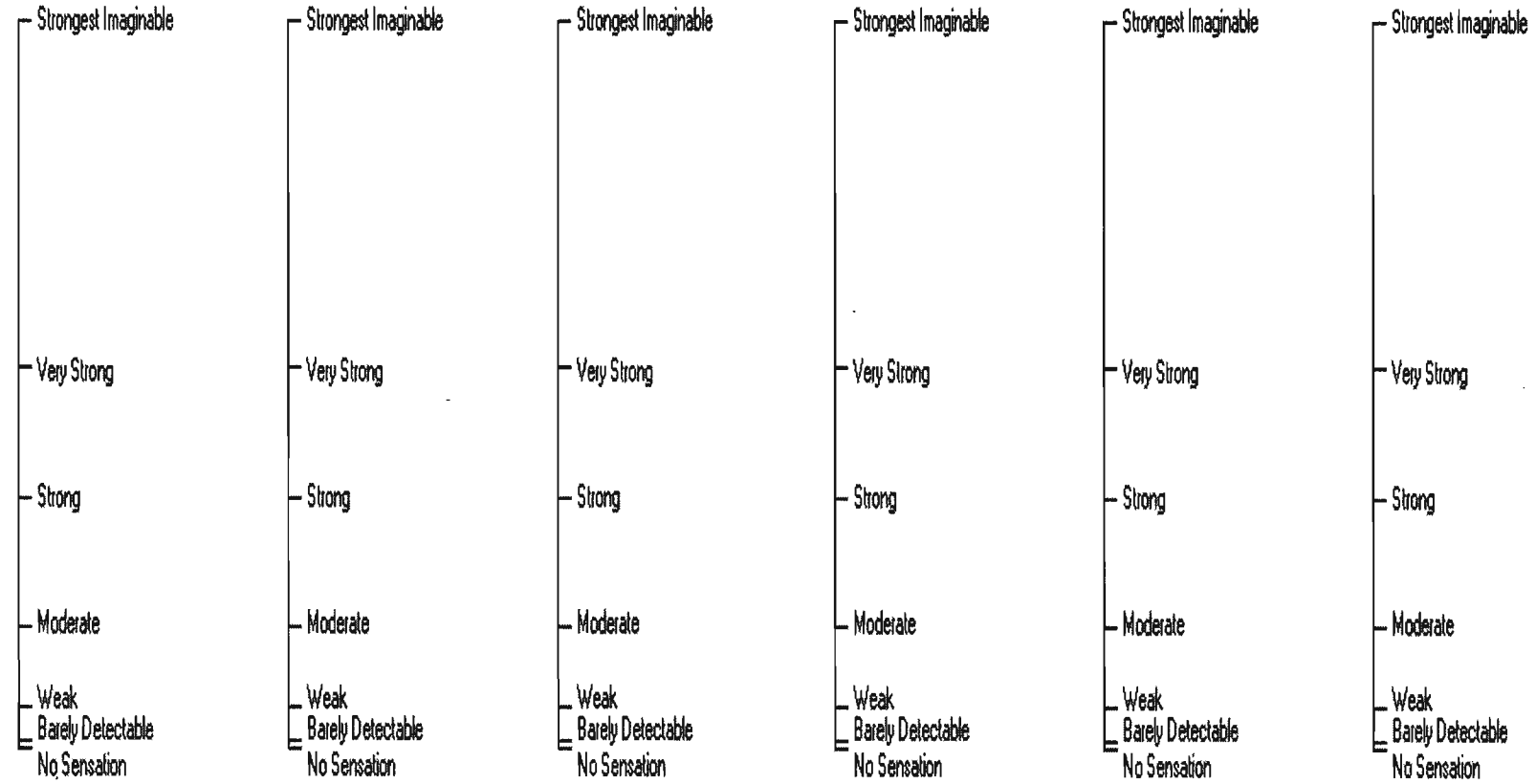
OTHER

SPECIFY:

You are being asked to rate the intensity the sensations you experienced upon heating of your tongue by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing.


Strongest Imaginable
Very Strong
Strong
Moderate
Weak
Barely Detectable
No Sensation
TEMPERATURE
SWEET
SALTY
SOUR
BITTER
OTHER
SPECIFY

APPENDIX D: DEMOGRAPHIC QUESTIONNAIRE FOR CHAPTER 3, 5, AND 6

Demographics

Age:

_____

Gender:

F ☐ M ☐

If you are a female, please indicate the first day of your last period (yymmdd).

_____

If you are no longer menstruating, please approximate the time since your last period (for weeks please include a 'w' after your answer, for months a 'm', and for years a 'y')

_____

Ethnicity (please check the one that best applies, if none of these apply, please indicate your ethnicity under 'Other'. If you would like to add any additional information, please do so under the 'Comments' section):

White	☐
Chinese	☐
South Asian	☐
Southeast Asian	☐
Black	☐
Filipino	☐
Japanese	☐
Latin American	☐
Arab	☐

Aboriginal ☐
(i.e., North American Indian/Metis/Inuit) ☐
Other (please specify): ☐

_____

Comments: _____

Were you born in North America:

Yes ☐ No ☐

If no, where were you born:

City: _____
Country: _____

How many countries outside of North America have you visited?

0 ☐
1-2 ☐
3-5 ☐
>5 ☐
>10 ☐

Do you exercise regularly:

Yes ☐ No ☐

If yes, how often:

_____ times/week

Do you regularly take vitamins or supplements:

Yes ☐ No ☐

Do you consume alcoholic beverages?

Yes ☐ No ☐

On average, how many times a month do you drink the following beverages?
(please check the appropriate box)

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20-24	25-29	>/=30
white wine																							
red wine																							
beer																							
spirits																							
other																							
(e.g. cooler)																							

Please indicate, on average, how many of the following beverages you consumer per day (on days when consuming alcoholic beverages).
(note: 1 drink = 12 oz. Beer = 6 oz. Wine = 1.5 oz. Spirit)

	#
white wine	
red wine	
beer	
spirits	
other	

(e.g. cooler) [redacted]

Do you smoke, or use tobacco products?

Yes ☐ No ☐

If yes, please indicate how many times a month you use the following tobacco products (please check the appropriate box)

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20-24	25-29	>/=30
cigarettes																							
cigars																							
pipe																							
snuff																							
chewing																							

Please indicate, on average, how many of the following tobacco products you use per day (on days when using tobacco products).

	#
cigarettes	
cigars	
pipe	
snuff	
chewing	

APPENDIX E: CHAPTER 6 NEOPHOBIA QUESTIONNAIRE

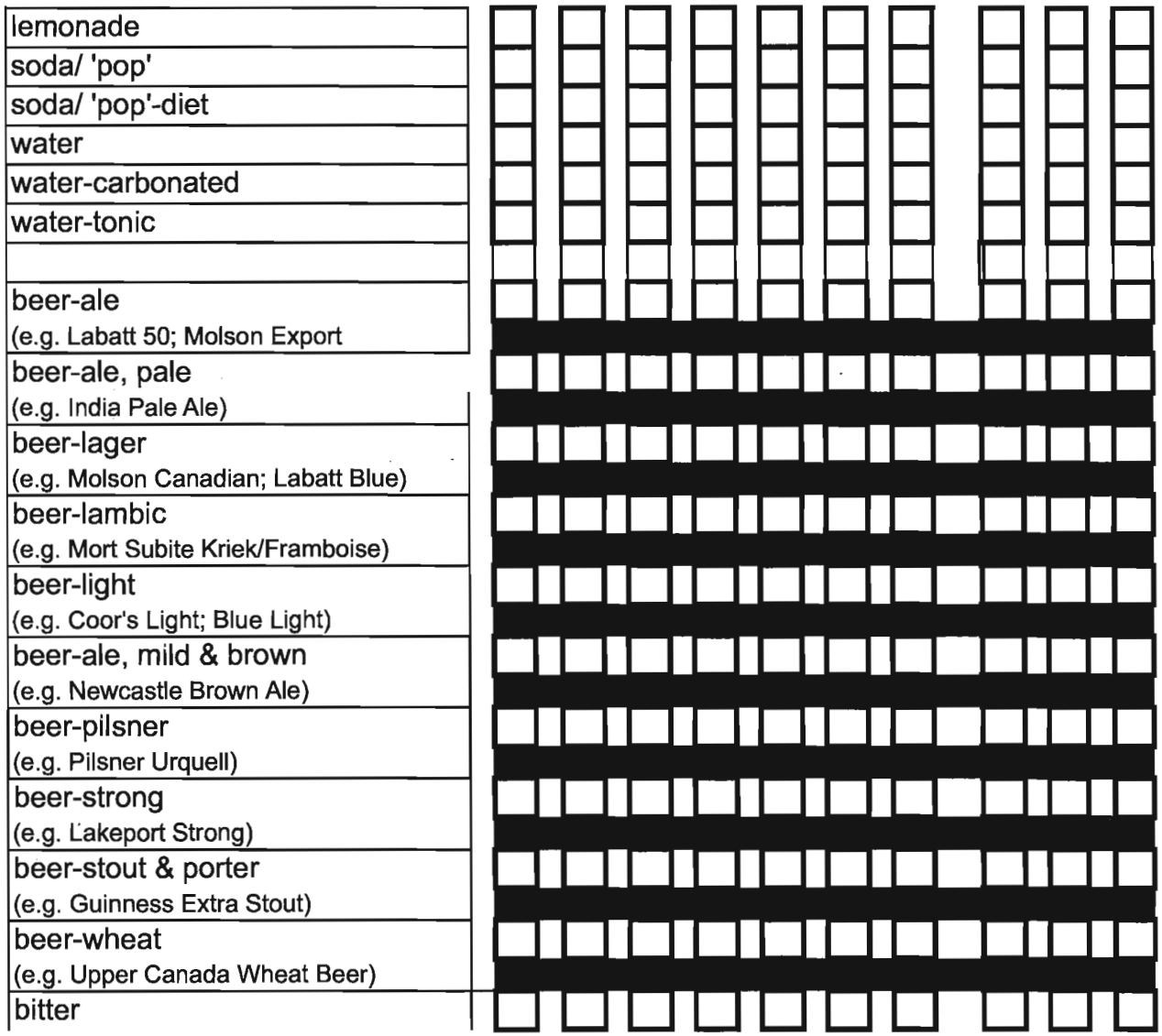
General Food Behaviours
Check the box next to each statement that best applies to you

	strongly agree			neither agree nor disagree			strongly disagree	can't answer
I am constantly sampling new and different foods/beverages	☐	☐	☐	☐	☐	☐	☐	☐
I don't trust new foods/beverages	☐	☐	☐	☐	☐	☐	☐	☐
I like foods/beverages from different countries	☐	☐	☐	☐	☐	☐	☐	☐
Ethnic foods/beverages look too weird to eat/drink	☐	☐	☐	☐	☐	☐	☐	☐
At dinner parties, I will try a new food	☐	☐	☐	☐	☐	☐	☐	☐
I am afraid to eat/drink things I have never had before	☐	☐	☐	☐	☐	☐	☐	☐
I will eat almost anything	☐	☐	☐	☐	☐	☐	☐	☐
I like to try new ethnic restaurants	☐	☐	☐	☐	☐	☐	☐	☐
I initiate trying new foods	☐	☐	☐	☐	☐	☐	☐	☐
I will try new foods/beverages when I am alone	☐	☐	☐	☐	☐	☐	☐	☐
I will try new foods/beverages if they are presented to me by someone I know	☐	☐	☐	☐	☐	☐	☐	☐
I feel pressure from others to try new foods/beverages	☐	☐	☐	☐	☐	☐	☐	☐

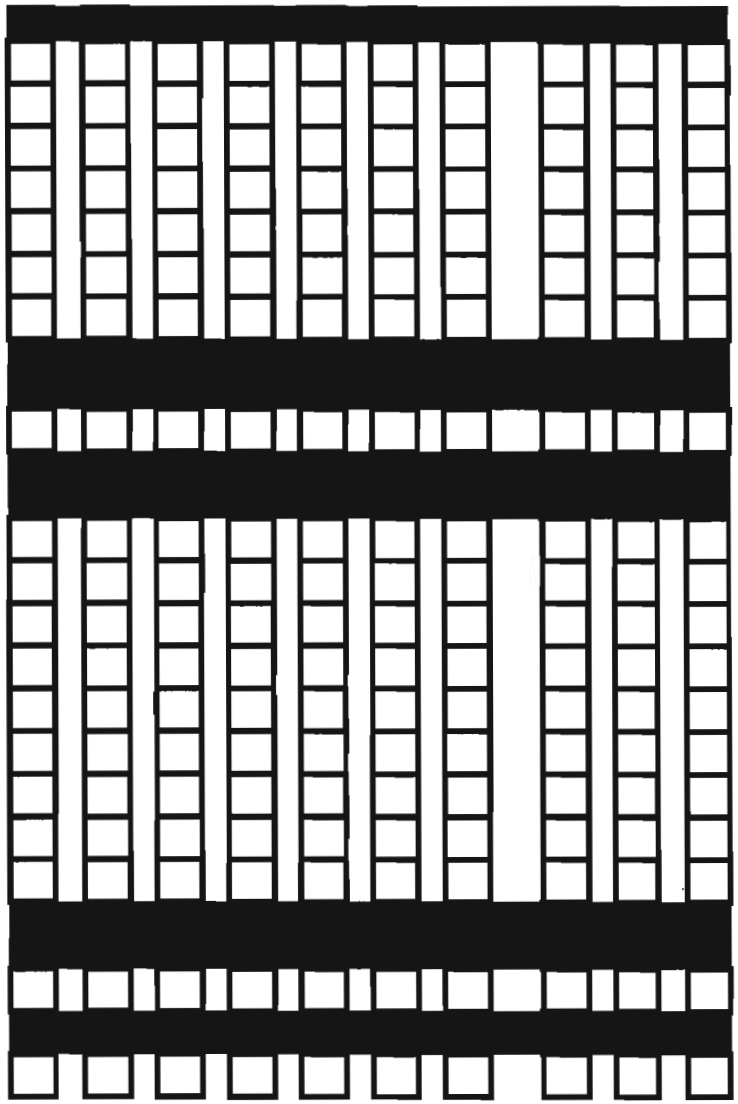
APPENDIX F: CHAPTER 6 FOOD QUESTIONNAIRE

Food Preferences
Check the box that best describes your overall liking of each of the following foods/beverages.
(Please check ONLY ONE box; if you are allergic to a food, but like/dislike it please check) the appropriate box AND the 'allergic' box

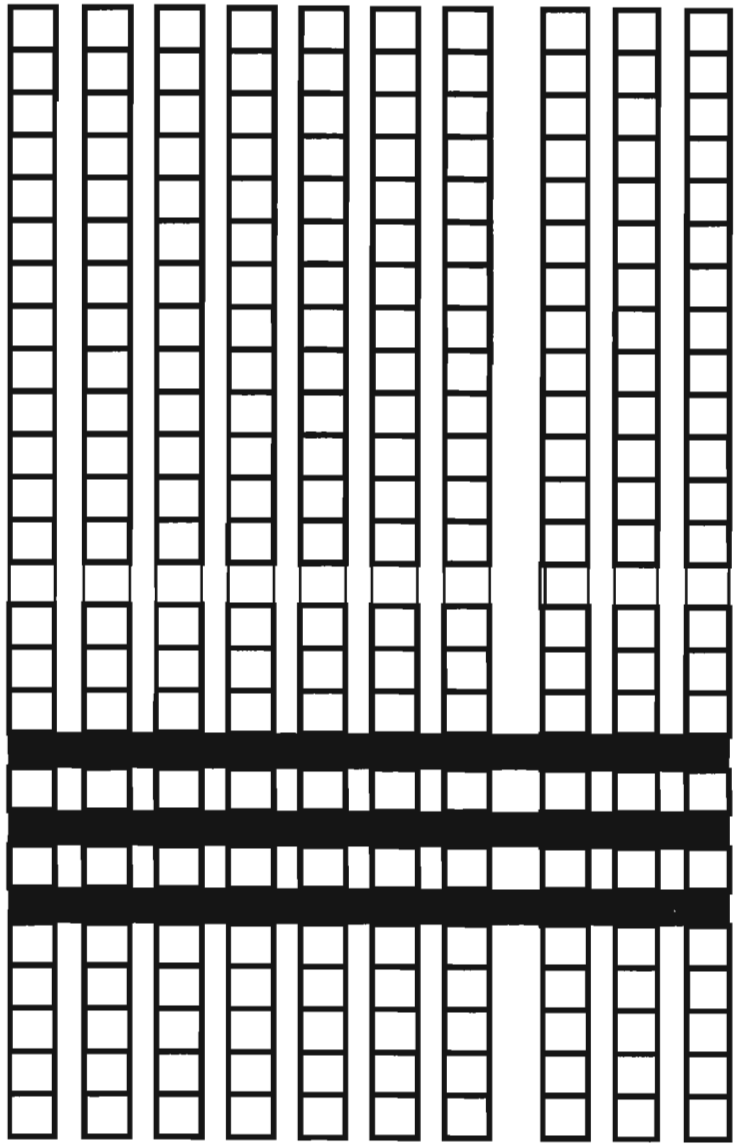
food/beverage	like extremely			neither like nor dislike			dislike extremely	allergic	never tried	don't know what is
cappuccino										
coffee-black										
coffee-milk/cream & sugar										
coffee-with milk/cream										
espresso										
hot chocolate										
tea-black										
tea-milk/cream & sugar										
tea-milk/cream										
tea-iced										
juice-fruit, clear										
juice-fruit, pulp										


lemonade
soda/ 'pop'
soda/ 'pop'-diet
water
water-carbonated
water-tonic
beer-ale
(e.g. Labatt 50; Molson Export
beer-ale, pale
(e.g. India Pale Ale)
beer-lager
(e.g. Molson Canadian; Labatt Blue)
beer-lambic
(e.g. Mort Subite Kriek/Framboise)
beer-light
(e.g. Coor's Light; Blue Light)
beer-ale, mild & brown
(e.g. Newcastle Brown Ale)
beer-pilsner
(e.g. Pilsner Urquell)
beer-strong
(e.g. Lakeport Strong)
beer-stout & porter
(e.g. Guinness Extra Stout)
beer-wheat
(e.g. Upper Canada Wheat Beer)
bitter

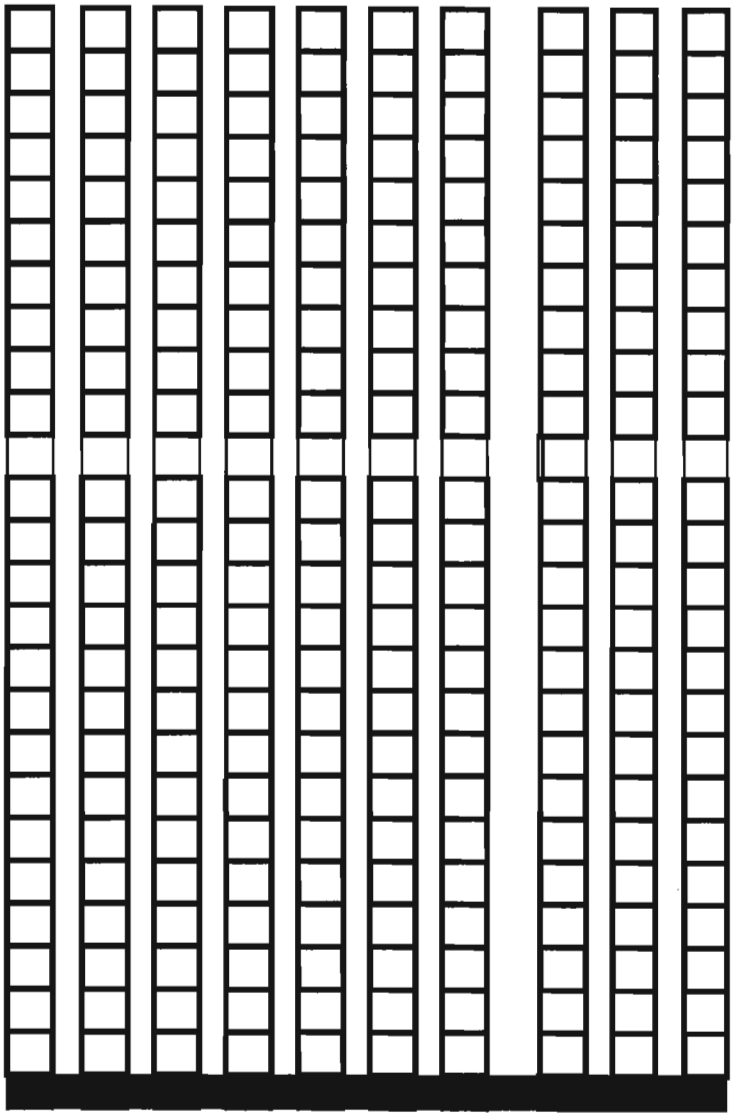
(e.g. Courage Directors Bitter)
bourbon
bourbon-mixed
brandy
cider
gin
gin-mixed
liqueurs-cream (e.g. Amarula, Baileys, Baja Rosa, Crème de Cacao, Crème de Banana,
liqueurs-clear (e.g. Triple Sec, Fruit Schnapps, Midori, Jagermeister, Sambuca)
port
rum
rum-cooler
rum-mixed
rye
rye-mixed
scotch
sherry
shots-mixed, bitter/sour/spicy (e.g. broken-down golf cart, prairie fire, brush fire, jagershock)
shots-mixed, sweet (e.g. B-52, lemon drop, tootsie roll)
tequila

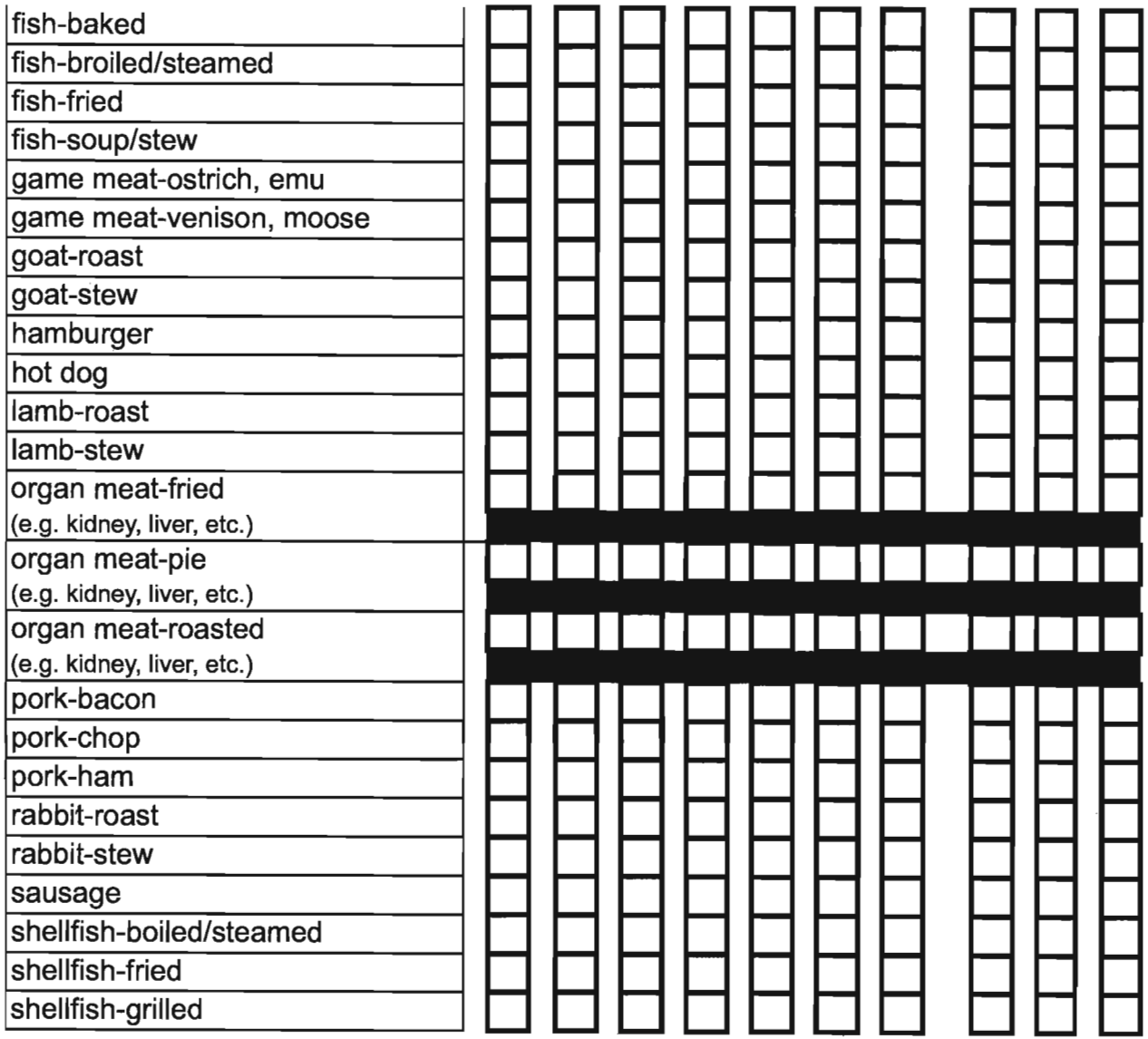


tequila-mixed
vodka
vodka-mixed
wine-cooler
wine-desert/ice
wine-dry sparkling
wine-fruit
wine-red dry
wine-red sweet
wine-rose/blush
wine-sweet sparkling
wine-white dry
wine-white sweet
cheese-cottage
cheese-cream
cheese-processed
(e.g. 'Kraft singles'
cheese-soft
(e.g., brie)
cheese-strong
(e.g., old cheddar)
cheese-feta
cream-10%
cream-table
cream-sour
cream-whipped

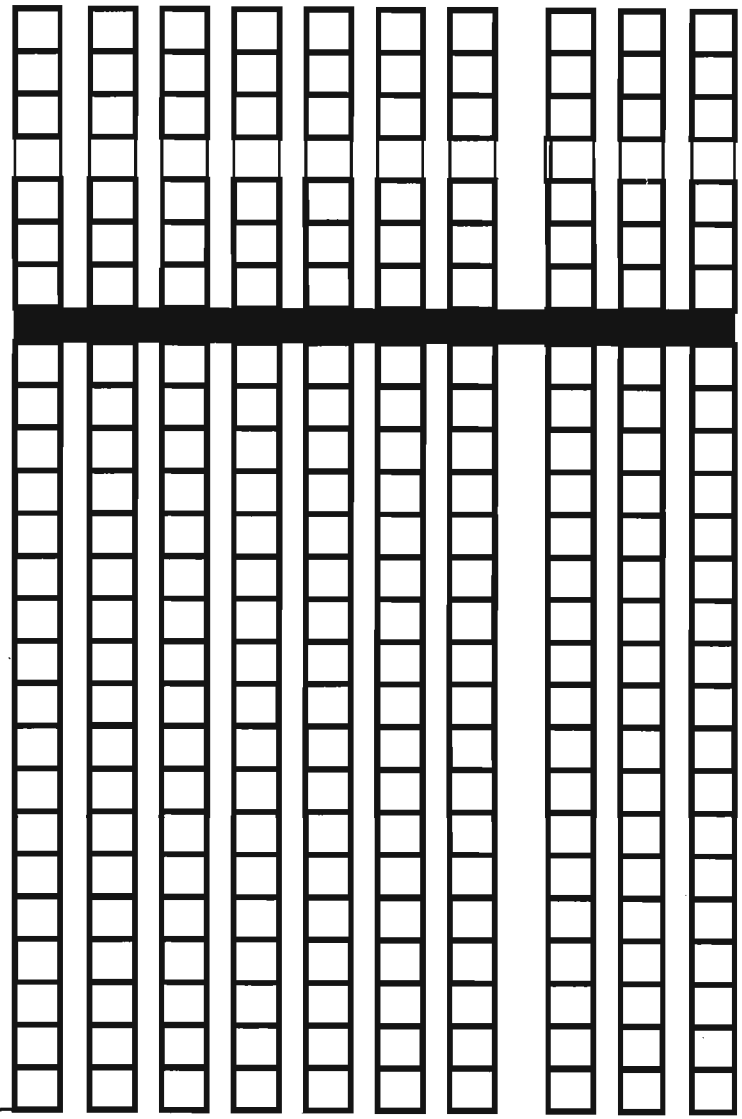


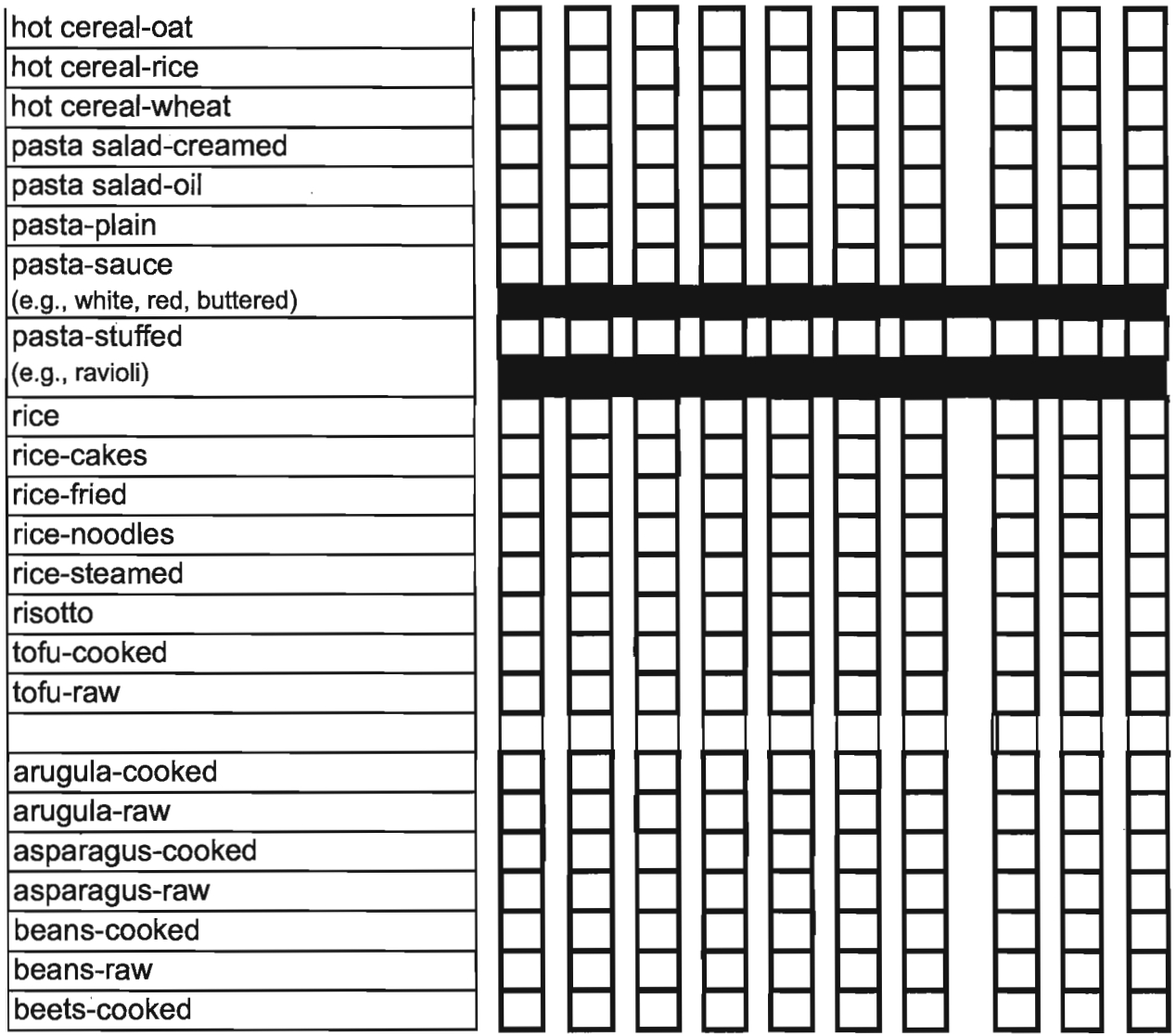
ice cream
milk -2%
milk-1%
milk-homo
milk-skim
milk-butter
milk-chocolate
milk-shake
yogurt-fruit-flavoured
yogurt-plain
beef-ribs
beef-roast
beef-steak
beef-stew
beef-veal
chicken-fried
chicken-grilled
chicken-roasted
egg-boiled
egg-fried
egg-poached
egg-salad (creamed)
egg-salad (oil)
eggs-prepared (e.g., deviled, pickled, quiche)

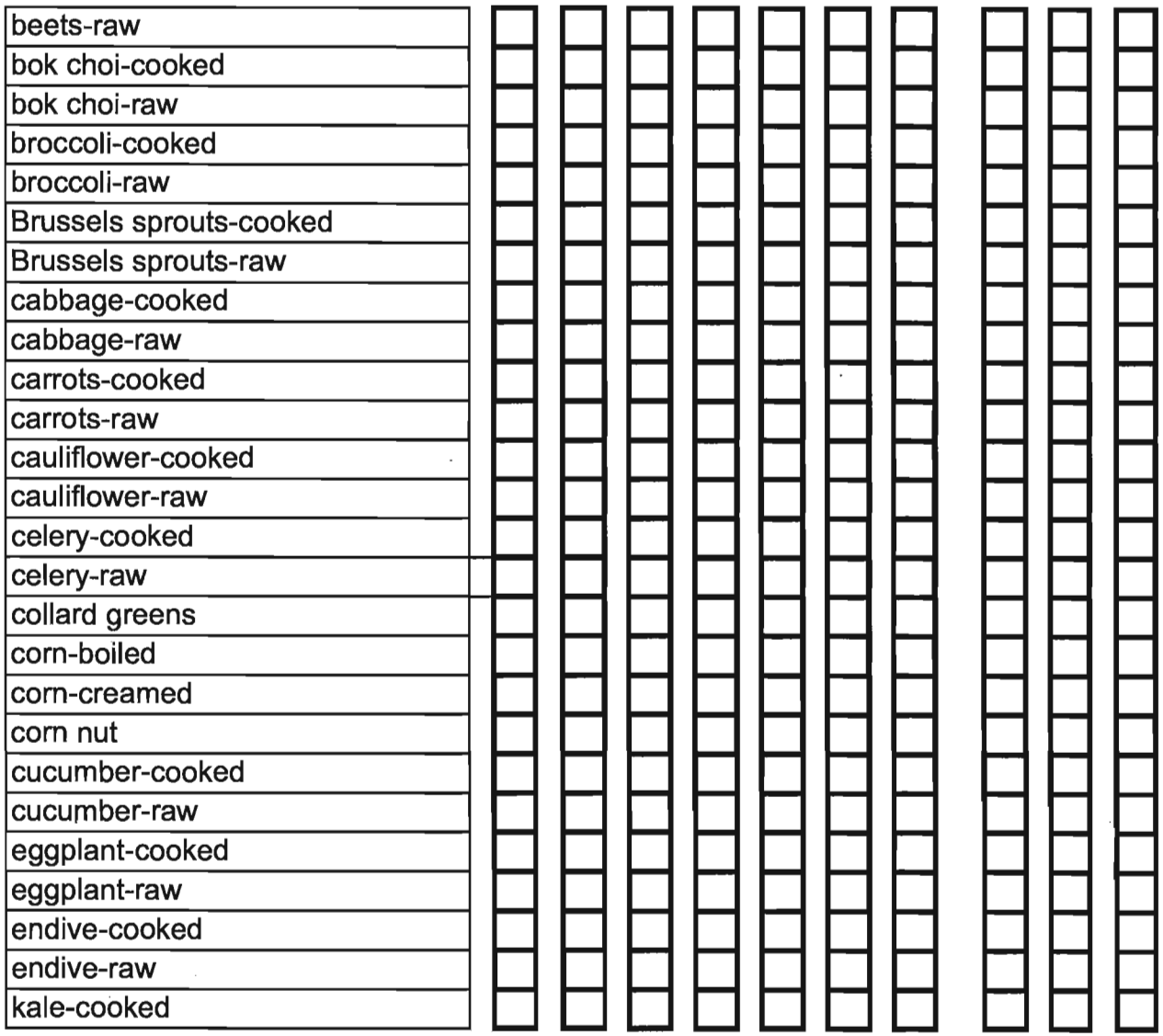



fish-baked
fish-broiled/steamed
fish-fried
fish-soup/stew
game meat-ostrich, emu
game meat-venison, moose
goat-roast
goat-stew
hamburger
hot dog
lamb-roast
lamb-stew
organ meat-fried
(e.g. kidney, liver, etc.)
organ meat-pie
(e.g. kidney, liver, etc.)
organ meat-roasted
(e.g. kidney, liver, etc.)
pork-bacon
pork-chop
pork-ham
rabbit-roast
rabbit-stew
sausage
shellfish-boiled/steamed
shellfish-fried
shellfish-grilled

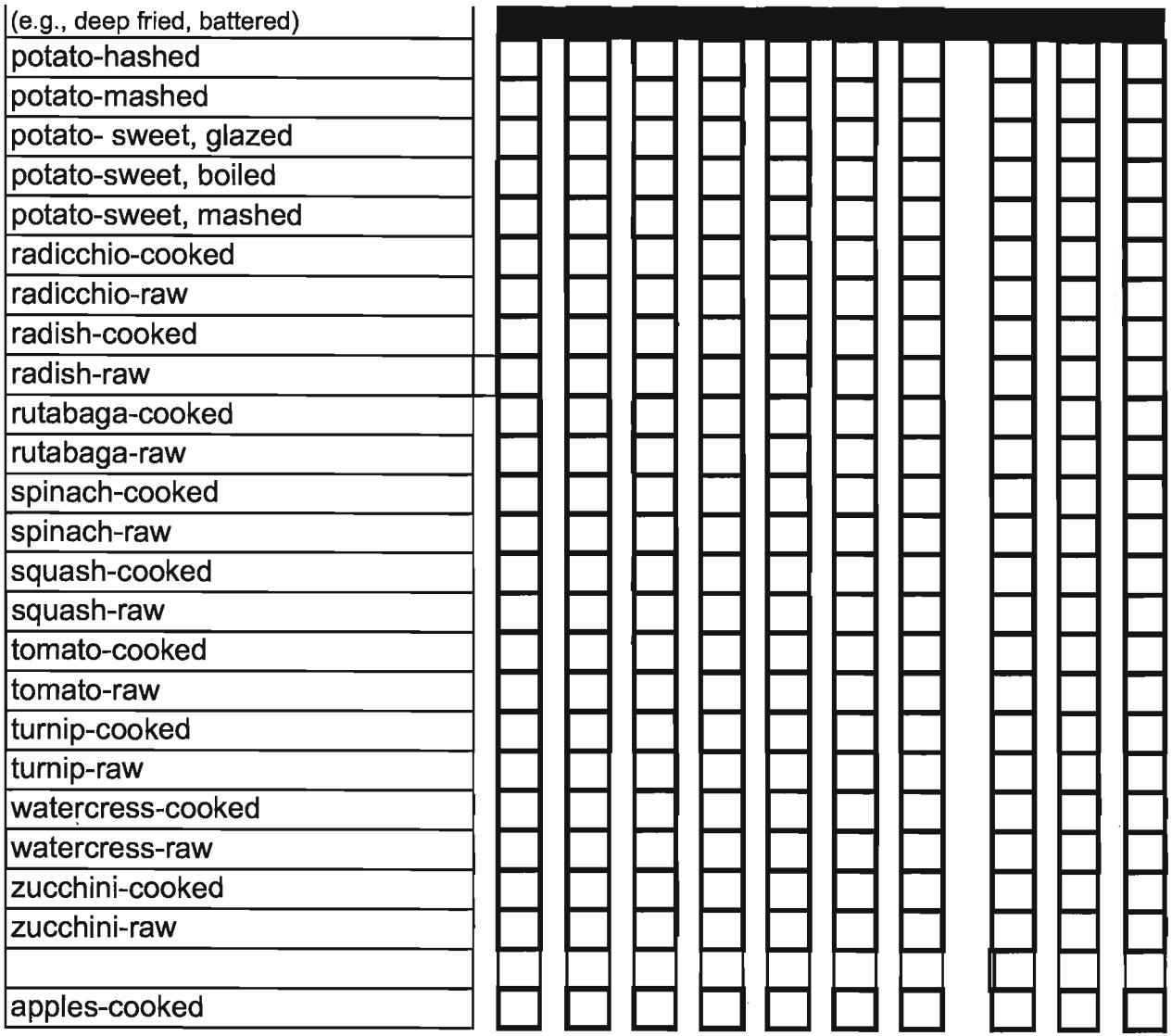
shrimp-boiled/steamed
shrimp-cocktail
shrimp-fried
barley
bread-brown/wheat
bread-other grain/seed (e.g., 12-grain, flax seed)
bread-pumpernickel
bread-sourdough
bread-sticks
bread-stuffing
bread-white
buckwheat
cold cereal-corn
cold cereal-oat
cold cereal-rice
cold cereal-wheat
cornbread
cornbread-stuffing
cornmeal
cracker-flavoured
cracker-plain
cracker-salted
croissants
hot cereal-corn



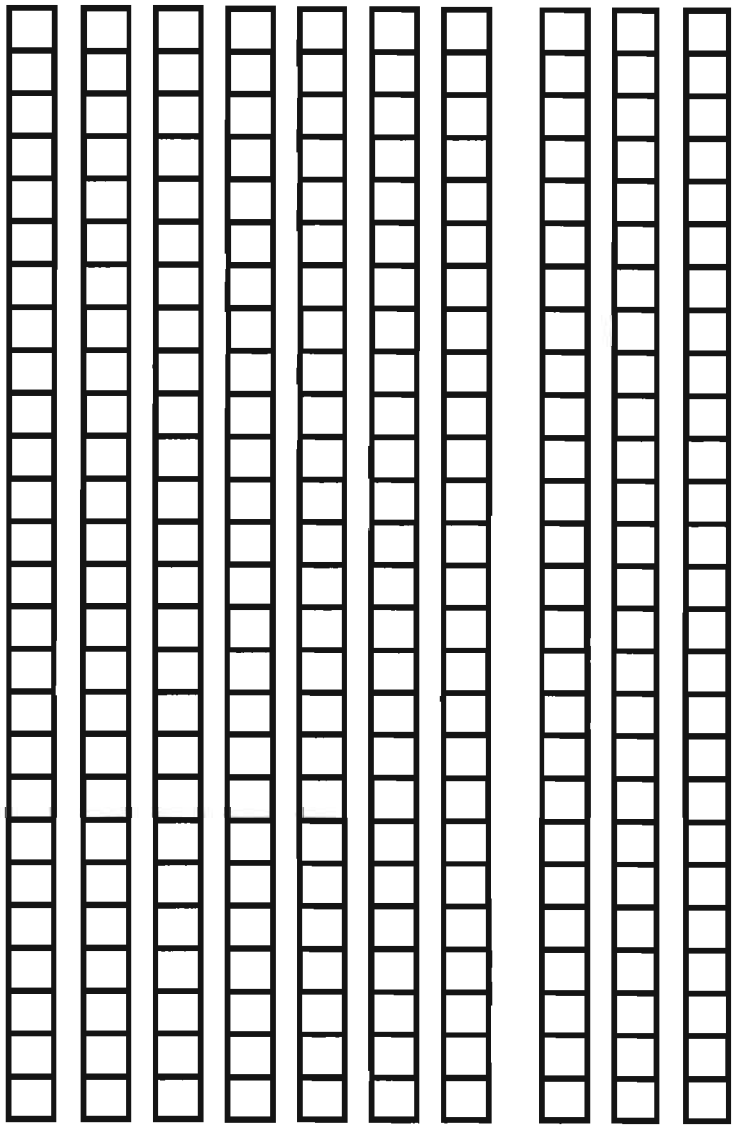

hot cereal-oat
hot cereal-rice
hot cereal-wheat
pasta salad-creamed
pasta salad-oil
pasta-plain
pasta-sauce
(e.g., white, red, buttered)
pasta-stuffed
(e.g., ravioli)
rice
rice-cakes
rice-fried
rice-noodles
rice-steamed
risotto
tofu-cooked
tofu-raw
arugula-cooked
arugula-raw
asparagus-cooked
asparagus-raw
beans-cooked
beans-raw
beets-cooked

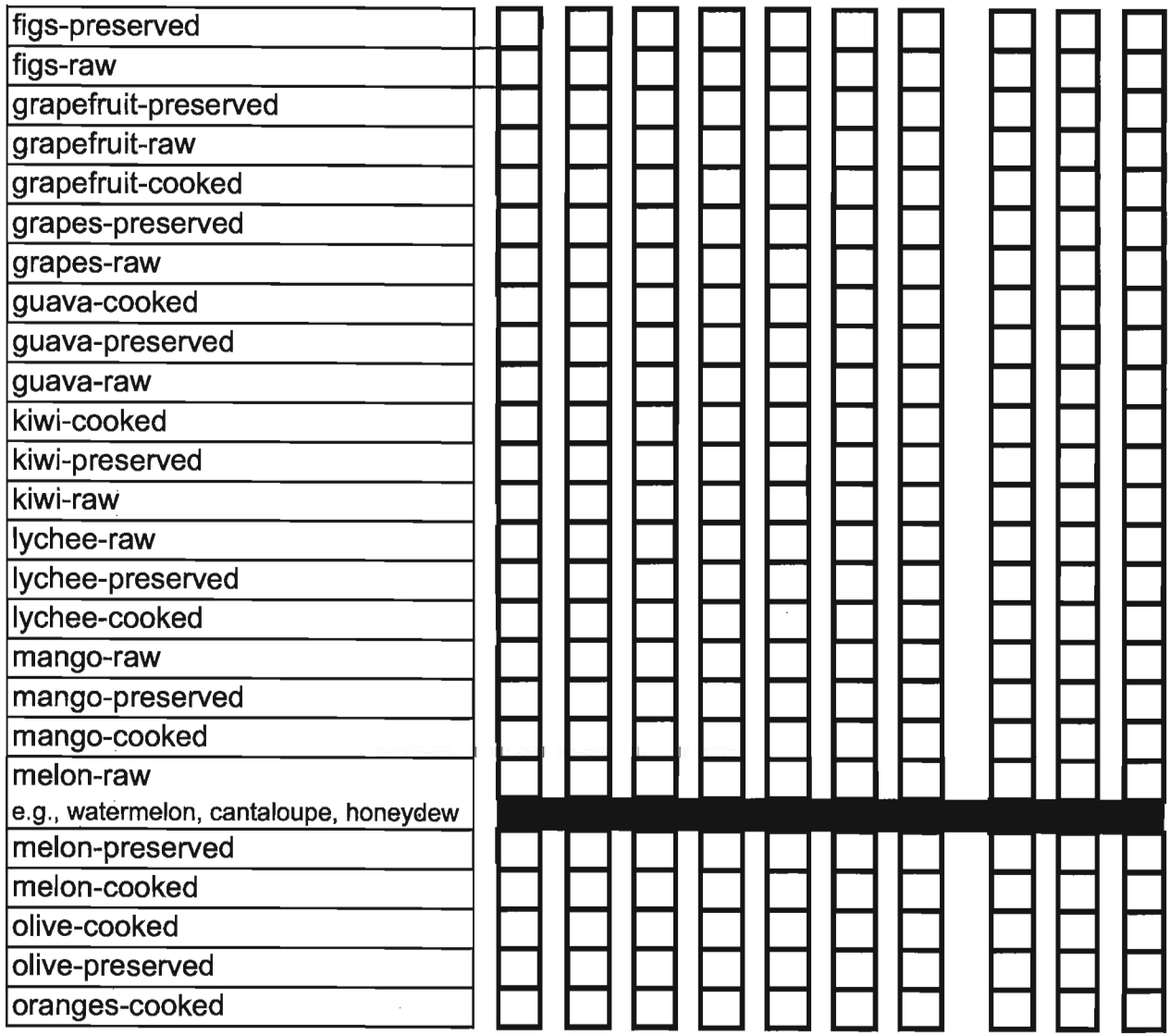

beets-raw
bok choi-cooked
bok choi-raw
broccoli-cooked
broccoli-raw
Brussels sprouts-cooked
Brussels sprouts-raw
cabbage-cooked
cabbage-raw
carrots-cooked
carrots-raw
cauliflower-cooked
cauliflower-raw
celery-cooked
celery-raw
collard greens
corn-boiled
corn-creamed
corn nut
cucumber-cooked
cucumber-raw
eggplant-cooked
eggplant-raw
endive-cooked
endive-raw
kale-cooked

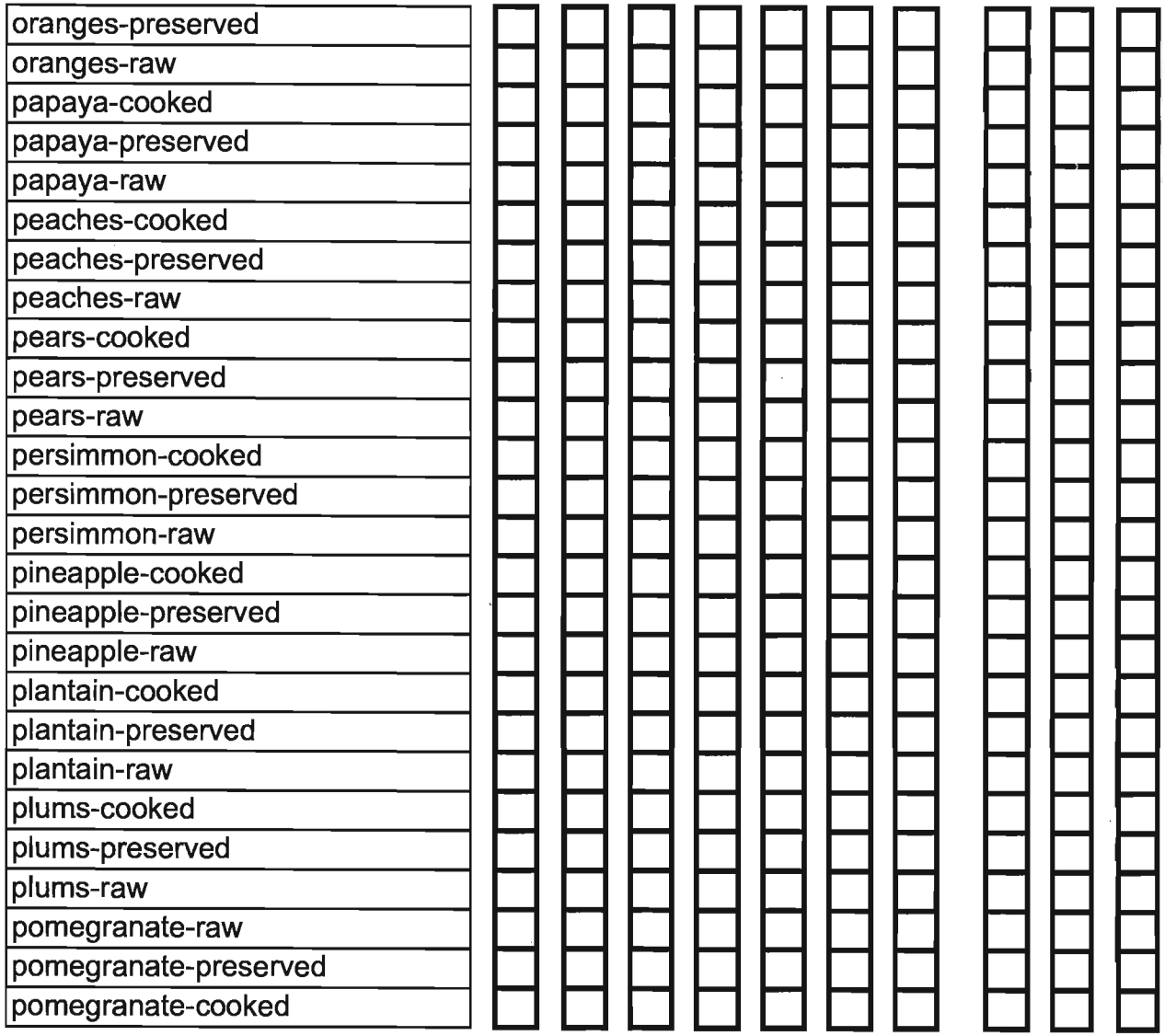
kale-raw											
kohlrabi-cooked											
kohlrabi-raw											
leek-cooked											
leek-raw											
lettuce-iceberg											
lettuce-romaine											
mushroom-cooked											
mushroom-raw											
mustard greens-cooked											
mustard greens-raw											
okra-cooked											
okra-raw											
onion-cooked											
onion-raw											
onion-rings											
peas-green, cooked											
peas-green, raw											
peppers (bell)-cooked											
peppers (bell)-raw											
peppers (hot)-cooked											
peppers (hot)-raw											
potato-boiled											
potato-salad											
potato-baked											
potato-fried											

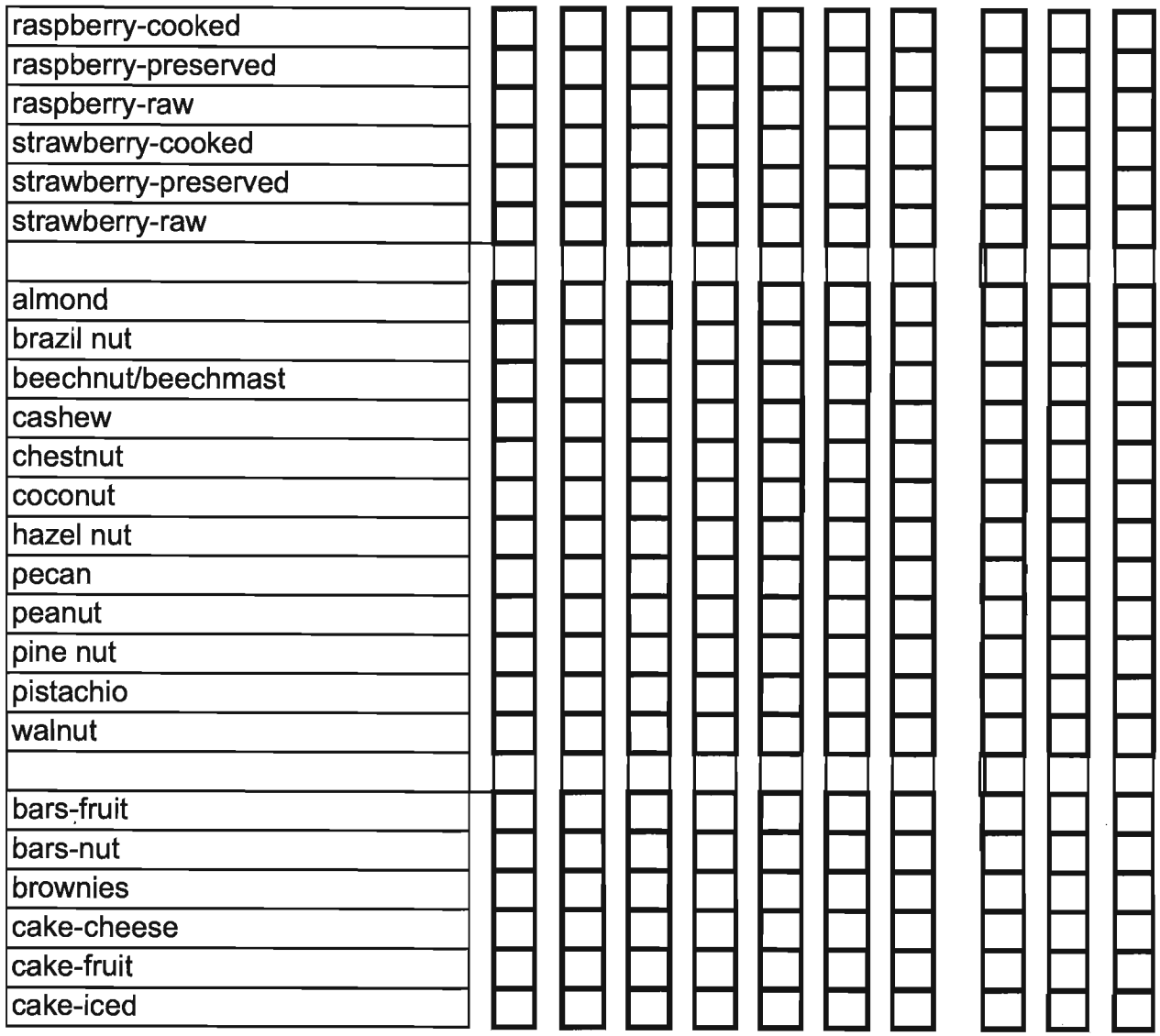

(e.g., deep fried, battered)
potato-hashed
potato-mashed
potato- sweet, glazed
potato-sweet, boiled
potato-sweet, mashed
radicchio-cooked
radicchio-raw
radish-cooked
radish-raw
rutabaga-cooked
rutabaga-raw
spinach-cooked
spinach-raw
squash-cooked
squash-raw
tomato-cooked
tomato-raw
turnip-cooked
turnip-raw
watercress-cooked
watercress-raw
zucchini-cooked
zucchini-raw
apples-cooked

apples-preserved
apples-raw
apricot-cooked
apricot-preserved
apricot-raw
avocado-cooked
avocado-preserved
avocado-raw
banana-cooked
bananas-raw
blackberry-cooked
blackberry-preserved
blackberry-raw
blueberry-cooked
blueberry-preserved
blueberry-raw
sweet cherry-cooked
sweet cherry-preserved
sweet cherry-raw
sour cherry-cooked
sour cherry-preserved
sour cherry-raw
currant-cooked
currant-preserved
currant-raw
figs-cooked

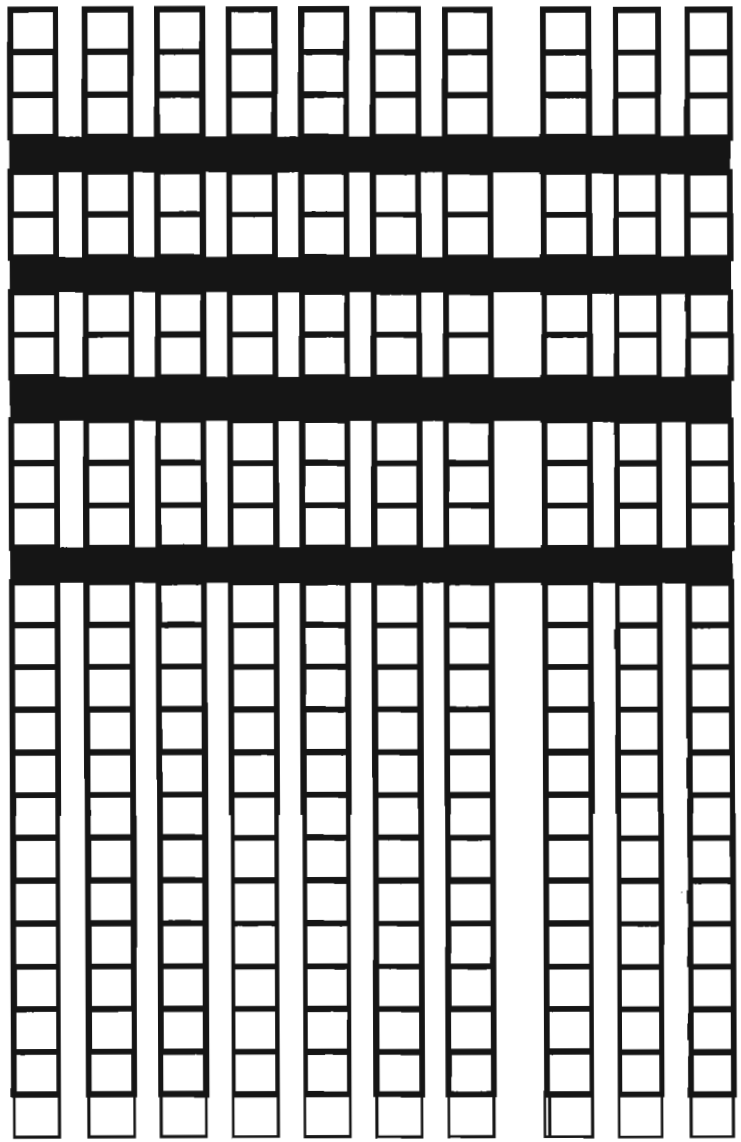


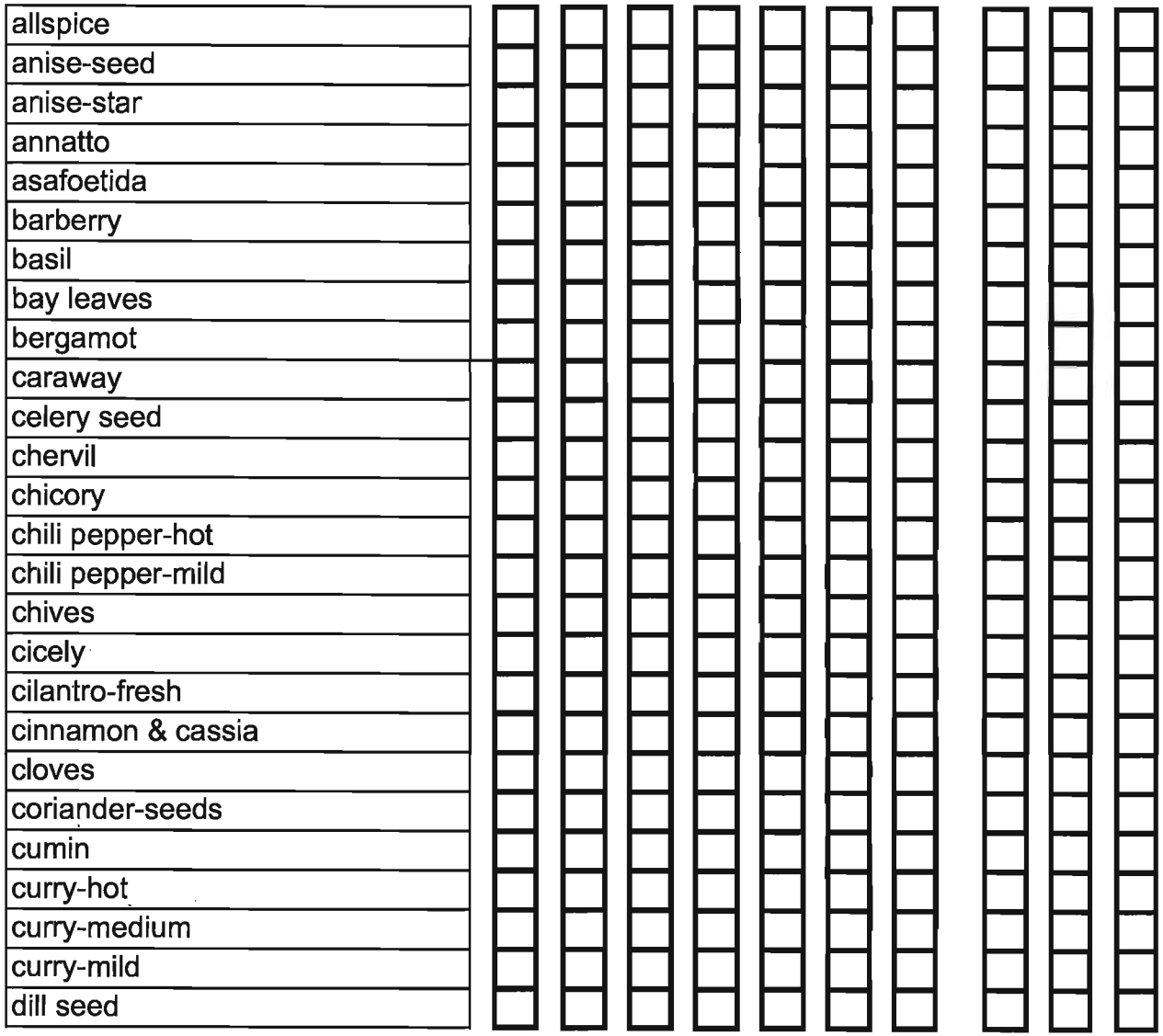

figs-preserved
figs-raw
grapefruit-preserved
grapefruit-raw
grapefruit-cooked
grapes-preserved
grapes-raw
guava-cooked
guava-preserved
guava-raw
kiwi-cooked
kiwi-preserved
kiwi-raw
lychee-raw
lychee-preserved
lychee-cooked
mango-raw
mango-preserved
mango-cooked
melon-raw
e.g., watermelon, cantaloupe, honeydew
melon-preserved
melon-cooked
olive-cooked
olive-preserved
oranges-cooked

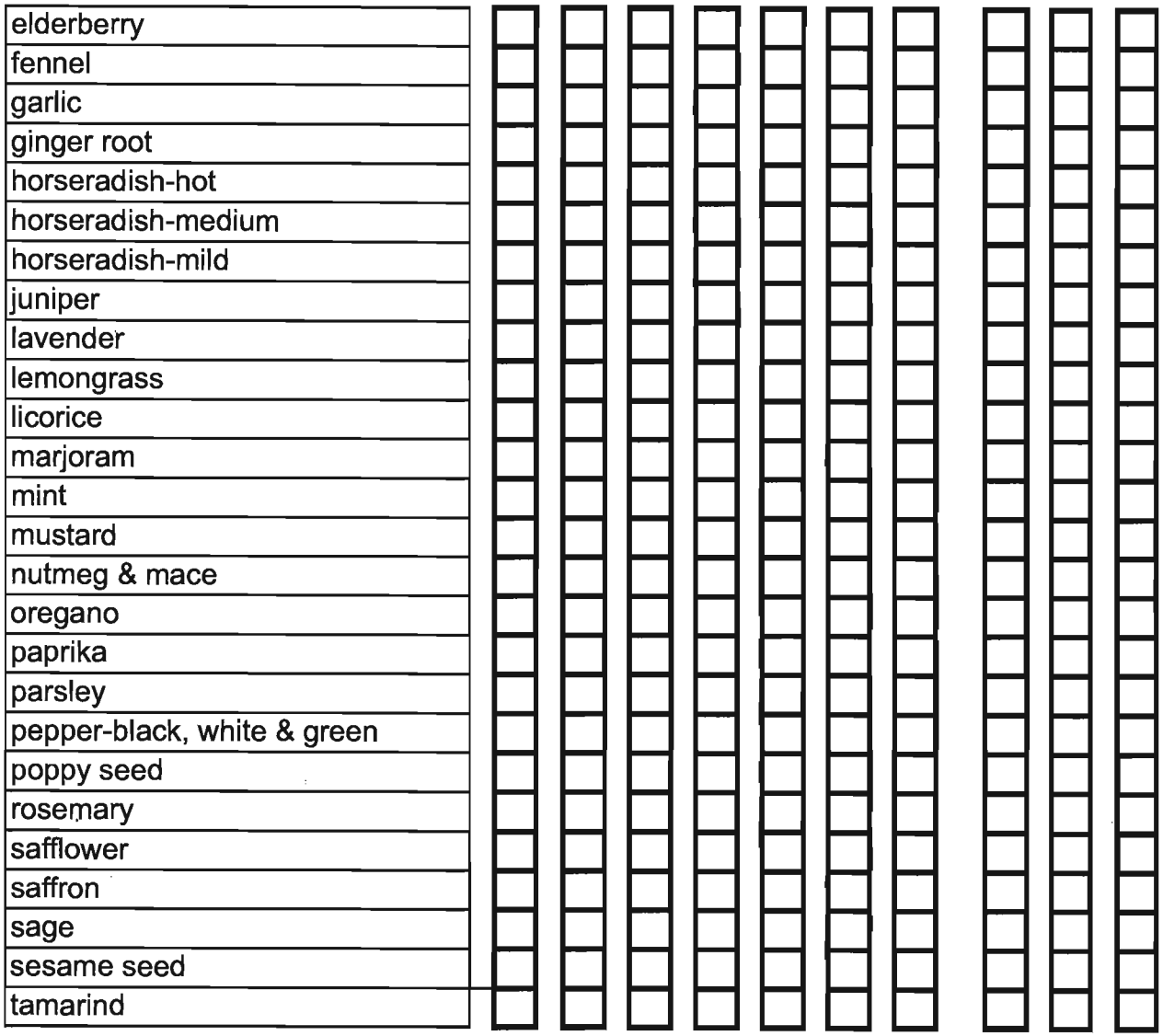

oranges-preserved
oranges-raw
papaya-cooked
papaya-preserved
papaya-raw
peaches-cooked
peaches-preserved
peaches-raw
pears-cooked
pears-preserved
pears-raw
persimmon-cooked
persimmon-preserved
persimmon-raw
pineapple-cooked
pineapple-preserved
pineapple-raw
plantain-cooked
plantain-preserved
plantain-raw
plums-cooked
plums-preserved
plums-raw
pomegranate-raw
pomegranate-preserved
pomegranate-cooked

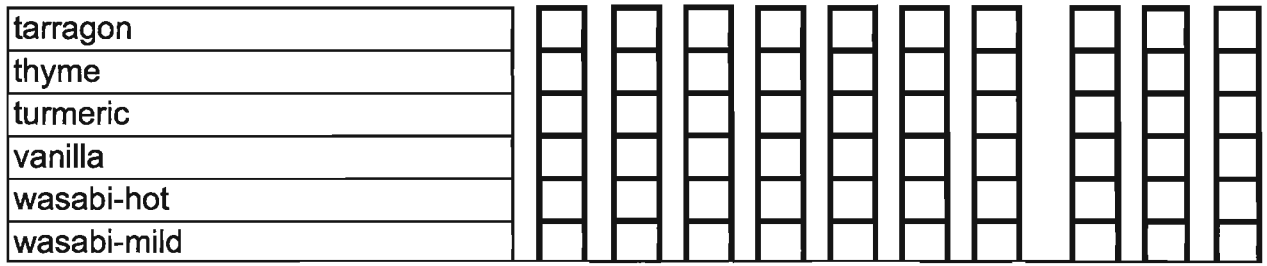

raspberry-cooked
raspberry-preserved
raspberry-raw
strawberry-cooked
strawberry-preserved
strawberry-raw
almond
brazil nut
beechnut/beechmast
cashew
chestnut
coconut
hazel nut
pecan
peanut
pine nut
pistachio
walnut
bars-fruit
bars-nut
brownies
cake-cheese
cake-fruit
cake-iced

cake-plain
cake-spice
cookies-decorated (e.g., sugared, glazed)
cookies-fruit
cookies-plain (e.g., biscuits, shortbread)
cookies-soft
cookies-spiced (e.g., gingerbread)
crisp-fruit
donut-plain
donut-fancy (e.g., glazed, decorated, filled)
gelatin-fruit
pie-cream
pie-deep fried
pie-fruit
pie-nut
pie-pudding
pie-pumpkin
pudding-flavoured
pudding-fruit
pudding-rice
strudel-fruit
strudel-other




allspice
anise-seed
anise-star
annatto
asafoetida
barberry
basil
bay leaves
bergamot
caraway
celery seed
chervil
chicory
chili pepper-hot
chili pepper-mild
chives
cicely
cilantro-fresh
cinnamon & cassia
cloves
coriander-seeds
cumin
curry-hot
curry-medium
curry-mild
dill seed


elderberry
fennel
garlic
ginger root
horseradish-hot
horseradish-medium
horseradish-mild
juniper
lavender
lemongrass
licorice
marjoram
mint
mustard
nutmeg & mace
oregano
paprika
parsley
pepper-black, white & green
poppy seed
rosemary
safflower
saffron
sage
sesame seed
tamarind


tarragon
thyme
turmeric
vanilla
wasabi-hot
wasabi-mild

APPENDIX G: CONSENT FORM FOR CHAPTER 4

INFORMATION STATEMENT AND CONSENT FORM

Name of Research Project: Temperature and thermal taste effects on time-dependent measures of oral sensation.

Department/Institute: Brock University; Department of Biological Science/ Cool Climate Oenology and Viticulture Institute

Principal Student Investigator: Martha Bajec, Ph.D. Candidate, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4719, mb96bm@brocku.ca

Principle Investigator/ Faculty Supervisor: Dr. Gary Pickering, Associate Professor, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4715, gpickeri@brocku.ca

Co-Investigator: Lynda VanZuiden, Research Assistant, Dept. of Biological Sciences, Brock University, (905) 688-5550 ext: 4719, lvanzuiden@brocku.ca

The Project:
The ability to taste the (potentially bitter and mildly unpleasant) compound 6-n-propylthiouracil (PROP), and the perceived intensity of this compound if detected, are largely genetically determined and may vary greatly from individual to individual. Sensitivity to PROP has been related to food preference and acceptance. Likewise, the ability to experience 'taste' sensations as a result of thermal stimulation has also been suggested to be genetically variable, and may have implications in food preference and acceptance. We have previously found that thermal tasters, those who perceive a taste from heating and cooling small areas of the tongue, perceive other oral stimuli (tastes, tactile sensations, and flavour) differently from thermal non-tasters. This project examines the effects of temperature and thermal taste on time-dependent parameters of the perception of oral sensations (e.g., time to maximum intensity, maximum intensity, duration, etc.).

The Procedure:
You are invited to participate in this study! A group of 40 participants that meet defined classification criteria will complete a general demographic and health questionnaire. These individuals will also have their PROP taster-status and thermal-taster status determined. PROP-taster status is determined by rating the intensity of a solution of PROP after orally rinsing with it (and expectorating). Quickly cooling and heating a small area of the tongue tip determines thermal-taster status. These participants will also use a method of measuring perceived intensity over time of sweet, sour, bitter, savory, and astringent stimuli at two different ecologically valid temperatures. Additionally, a

sample of DNA will be taken in a non-invasive procedure (you will rinse with a commercial mouthwash and spit into a sample cup) and analyzed to examine the genes involved in thermal taste.

Benefits/Risks

The expected benefits for the participant and the scientific community are a greater understanding of the role of genetically-mediated indices in taste perception. The determination of thermal taster status is very novel, as such, participants will be among a very few others (less than 300 people world-wide) who have had their thermal-taste status determined, and the first to have their DNA analyzed for this source of individual variation. In addition, participants will gain awareness of their palates, and potentially food preferences.

The expected risks are no great than those encountered in normal daily food and beverage consumption. While they may not be pleasant to everyone, all substances to be tasted are perfectly safe, and are of food-quality grade or better.

Those participants that meet the defined classification criteria will receive a $25 gift certificate for the Brock University Book Store, or Chapter's, or The Pen Center, or The Fairview Mall, as requested.

Voluntary Participation:

You are free to withdraw your participation in the research at any time, and if you do, any data collected from will immediately be destroyed.

Responsibilities:

The participant needs only to schedule 6, 1-hour blocks of time to come to the CCOVI Sensory Lab (IH301) at Brock University. Times will be available during normal working hours, after working hours, and on weekends, as agreed upon by the participant and the Principle Student Investigator.

Publication of Results

It is expected that the results of this study will be published, in academic journals and presented at conferences. Please feel free to contact either the Principle Student Investigator, or the Principle Investigator/Faculty Supervisor at any time should any questions arise. Also, for more information on the progress or results of the study please contact either persons mentioned above, or consult the Pickering lab website at:
http://www.brocku.ca/ccovi/pages/people/show.php?id=9.

Confidentiality

All data will be confidential, individual identities will not be disclosed to anyone outside of the researchers listed above. Samples (i.e., DNA) collected will be destroyed within 24 months of collection. Paper data collected in this study (i.e., questionnaires) during this study will be will be retained for 7 years. Digitally

recorded measures (i.e., intensity measures collected via computer) will be retained on disc for 7 years. Data will be stored in a locked, private area accessible only to the Principle Investigator/Faculty Supervisor. Only the individuals listed above will have access to the data.

Ethics Clearance:

This study has been reviewed and received ethics clearance through the Research Ethics Board at Brock University (file 08-006 Pickering/Bajec). If you have any comments or concerns about your rights as a research participant, please contact the Research Ethics Office at (905) 688-5550 Ext. 3035, reb@brocku.ca.

Consent:

The purpose of the research has been explained to me, including the potential risks/discomforts associated with the research. I have also been given the opportunity to ask questions about the research and received satisfactory answers, and know that I may continue to ask questions and receive satisfactory answers throughout the study.

I understand that I am free to withdraw my participation in the research at any time and that if I do I will not be subjected to any penalty or discriminatory treatment; however, I do understand that my name will be withdrawn from the gift certificate draw. I also understand my participation in this project is on a voluntary basis, and no remuneration will be provided by Brock University in exchange for my participation.

I understand that any information or personal details gathered in the course of this research about me are confidential and that neither my name nor any other identifying information will be used or published without my written permission.

This study has been reviewed and approved by the Brock University Research Ethics Board (Ethics File Number 08-006 Pickering/Bajec). I understand that if I have any complaints or concerns about this research I can contact:
Research Ethics Officer, Office of Research Services, Brock University, Ph: 905 688 5550, ext: 3035; **reb@brocku.ca**

Your Name: _____

Signature: _____

Date: _____

Please check the box below if you **DO NOT** wish to give a DNA sample.

☐

Please check the box below if you **ARE NOT** interested in being contacted to participate in the future studies performed by the Pickering lab.

☐

Please check the store for which you would like to receive a $25 gift certificate:

☐ The Brock University Bookstore

☐ Chapter's

The Pen Center ☐

The Fairview Mall ☐

APPENDIX H: CHAPTER 4 TASTING INSTRUCTIONS

Tasting Protocol – please follow these instructions when tasting your samples

1) before starting, **rinse** well with **water**

2) take the **entire volume** of the sample into your mouth, **rinse gently** for **five (5) seconds**

3) **spit out** the sample and **wait ten (10) seconds**

4) **rate** the **maximum intensity** experienced **during** the **entire previous fifteen (15) seconds** (i.e., from the start of step 2 to the end of step 3) on the scale provided

5) **rinse once** with **pectin**

6) **rinse** at least **twice** with **water**

7) **wait** at least **one (1) minute** before going on to the next sample, or as long as it takes to **extinguish** the **taste** from the **pervious sample; feel free to rinse more and take more time, if needed**

APPENDIX I: CHAPTER 4 TIME-INTENSITY TASTING PROTOCOL

Instructions:

You are being asked to rate the intensity of the sample provided over time. This task requires you to *move the mouse up and down* on the line scale in accordance with the intensity of the taste you perceive in your mouth. You ***must rate the sample immediately upon putting it in your mouth, through spitting it out, and continuously until the taste is no longer perceivable*** to you. If the *taste is extinguished*, you may click on 'NS' (No Sensation) and the test will end for that sample. You will take a 2-minute break before you receive your next sample. In that *2-minute break, please rinse your mouth with pectin then rinse at least 2 times with water*. Once your 2-minute break is over, *please open the hatch in your booth*.

The step you should follow are:
1) place the ***sample at lips***, ***click*** on ***start*** and ***immediately take entire volume in mouth***

2) ***rinse gently*** with the sample ***until*** the screen tells you to ***"Spit it out"***

3) ***continue rating*** the intensity, *keeping your mouth closed and motionless (you may move your tongue, but try not to make chewing motions with your jaw, or open your mouth)*, ***until the taste is gone*** (if intensity reaches zero, click on 'NS' to escape)

4) open hatch door

5) rinse with the solutions provided:
-once with pectin, and twice with water

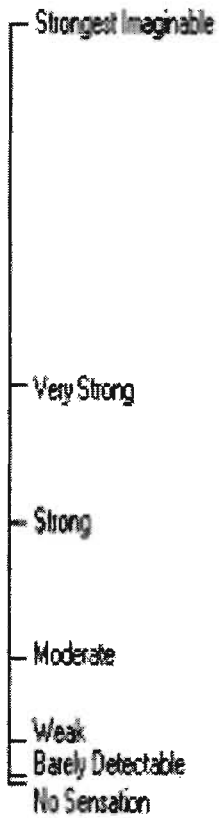
APPENDIX J: CHAPTER 4 SCALES

You are being asked to rate the intensity of a remembered sensation, namely, the **sweetness of a banana**, by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing. *Please mark the scale with a horizontal line only.*

sweetness of a banana

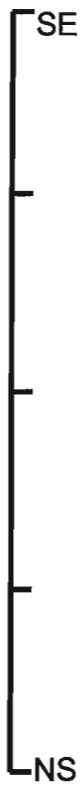


You are being asked to rate the intensity of a remembered sensation, namely, the **sweetness of a banana,** by indicating where it lies on a scale of all experienced sensations. Please take a moment to think about the last time you experienced this sensation, and how intense it was for you.

The top of the scale (*SE*) is the *Strongest sensation of any kind that you have ever Experienced*, which includes pain. The bottom of the scale (*NS*) is *No Sensation*.

Please mark the scale with a horizontal line only.

sweetness of a banana



OTHER REMEMBERED SENSATIONS RATED

-bitterness of black coffee
-brightness of the sun
-burn of cinnamon gum
-coolness of an ice-cold beverage
-sweetness of cotton candy
-burning sensation from eating a whole hot pepper
-heat of drinking a hot tea
-warmth of sipping lukewarm water
-pain from biting your tongue
-coolness of a peppermint candy
-touch sensation of a pill on your tongue
-saltiness of ocean water
-sourness of a lemon
-tingling from a carbonated beverage

Please rinse with the water provided. You are being asked to rate the intensity of **astringency** by indicating where it lies on a scale of all experienced sensations. The top of the scale (*SE*) is the *Strongest sensation of any kind that you have ever Experienced*, which includes pain. The bottom of the scale (*NS*) is *No Sensation*.

Please take the entire volume of the sample provided, swish it around in your mouth for five (5) seconds, then expectorate (i.e., spit out). After you have expectorated, wait approximately ten (10) seconds and then rate the maximum intensity that you perceived in the preceding fifteen (15) seconds. Please keep in mind, that you are rating the **maximum intensity** for astringency, whenever it may occur.

Please mark the scale with a horizontal line only.

astringency

Please follow the sampling instructions found on the rating page.

The five glasses in front of you each contain one of the stimuli you just sampled. Select the identity of the sample from the list below and note it on your rating sheet as 'sample ID:'

Astringent
Bitter
Sour
Sweet
Umami/savoury

You are being asked to rate the intensity of the sample by indicating where it lies on a scale of all experienced sensations. The top of the scale (*SE*) is the *Strongest sensation of any kind that you have ever Experienced*, which includes pain. The bottom of the scale (*NS*) is *No Sensation*.

Please take the entire volume of the sample provided, swish it around in your mouth for five (5) seconds, then expectorate (i.e., spit out). After you have expectorated, wait approximately ten (10) seconds and then rate the maximum intensity that you perceived in the preceding fifteen (15) seconds. Please keep in mind, that you are rating the **maximum intensity** for the sample, whenever it may occur.

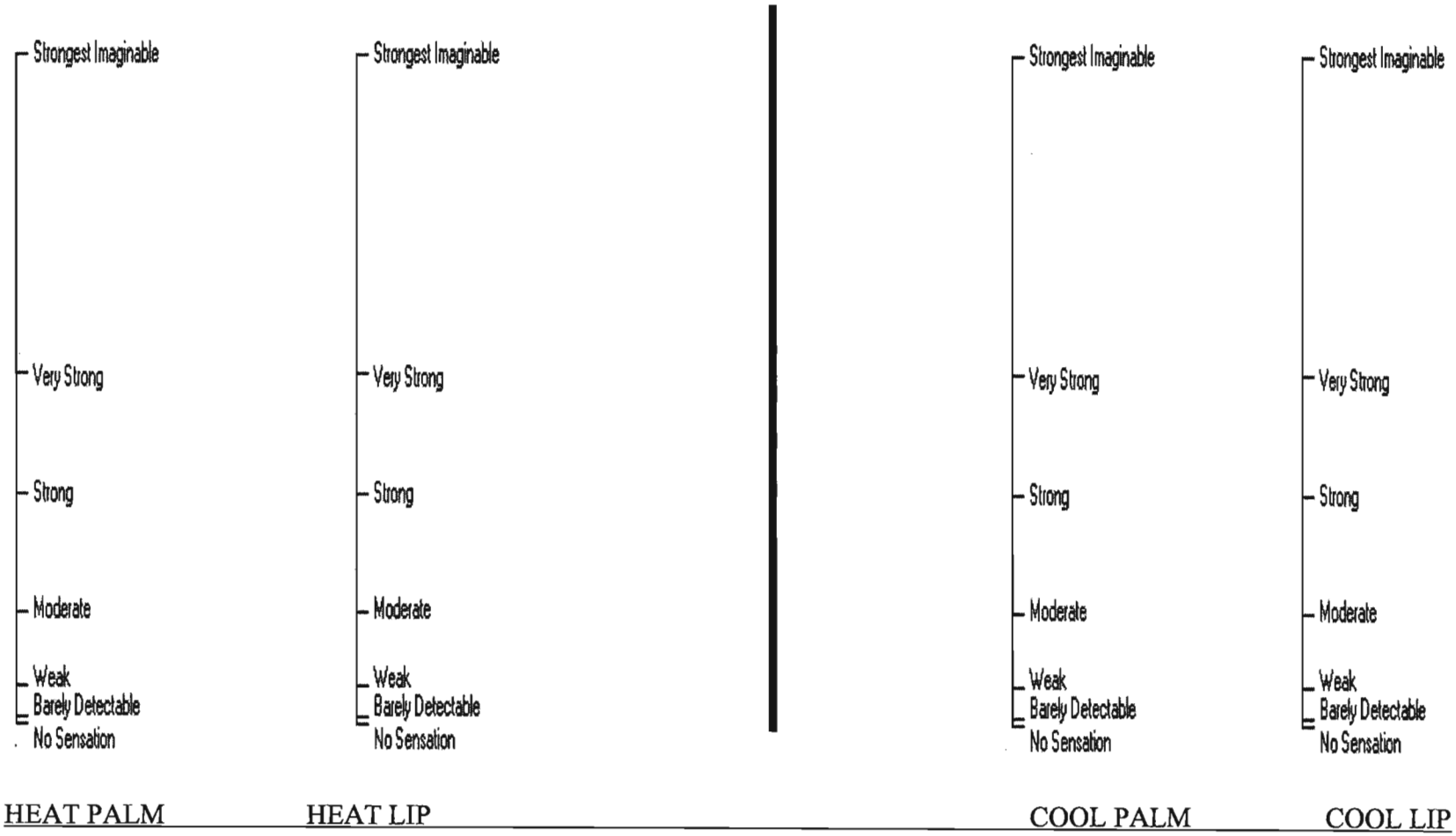
Please mark the scale with a horizontal line only.

sample ID: _____

You are being asked to rate the intensity of the **temperature applied to your lip, and the temperature applied to the palm of your hand** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

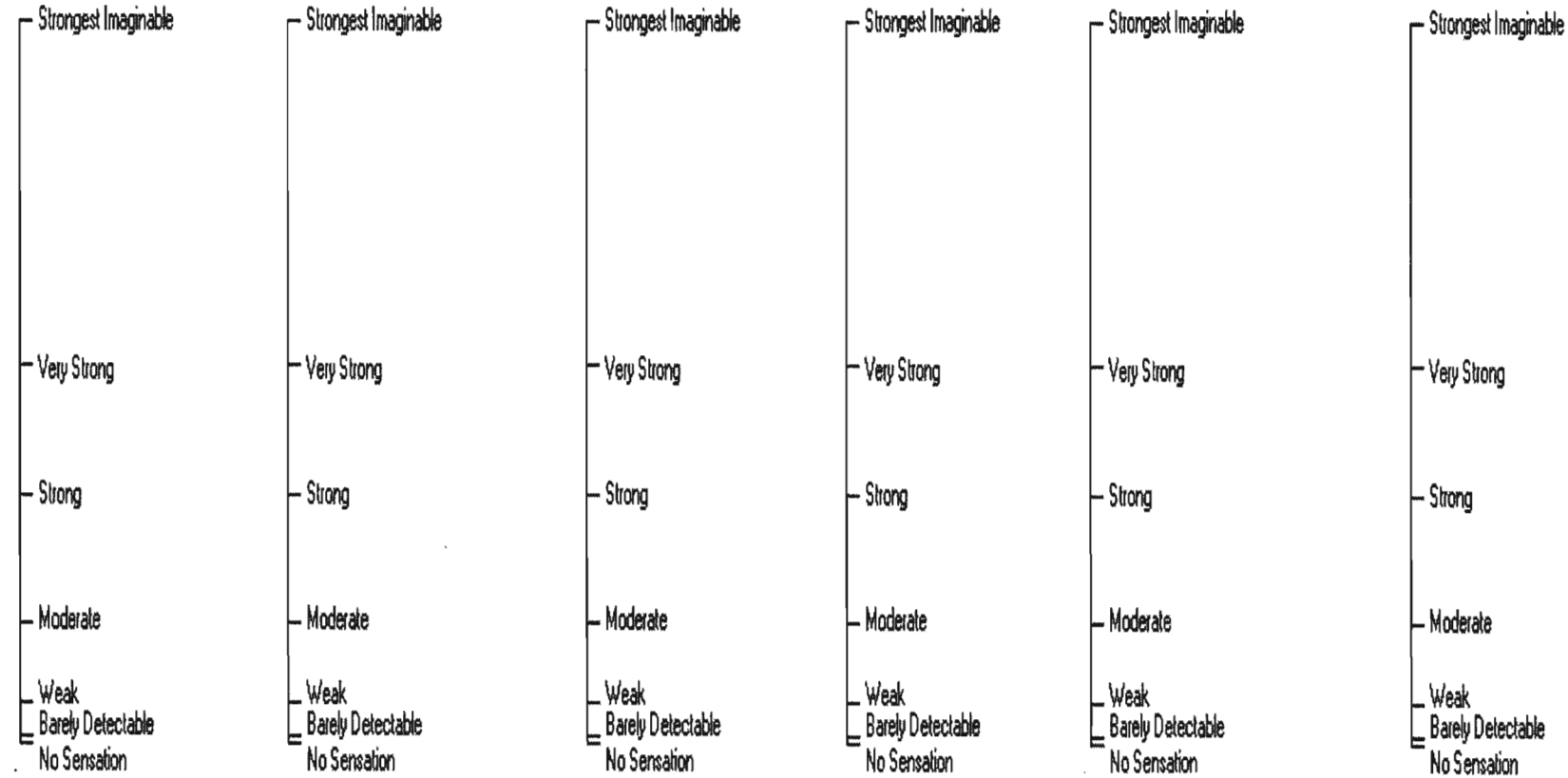
It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing. *Please mark the scale with a horizontal line only.*


Strongest Imaginable
Very Strong
Strong
Moderate
Weak
Barely Detectable
No Sensation
HEAT PALM
Strongest Imaginable
Very Strong
Strong
Moderate
Weak
Barely Detectable
No Sensation
HEAT LIP
Strongest Imaginable
Very Strong
Strong
Moderate
Weak
Barely Detectable
No Sensation
COOL PALM
Strongest Imaginable
Very Strong
Strong
Moderate
Weak
Barely Detectable
No Sensation
COOL LIP

You are being asked to rate the intensity the sensations you experienced upon **cooling of your tongue** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

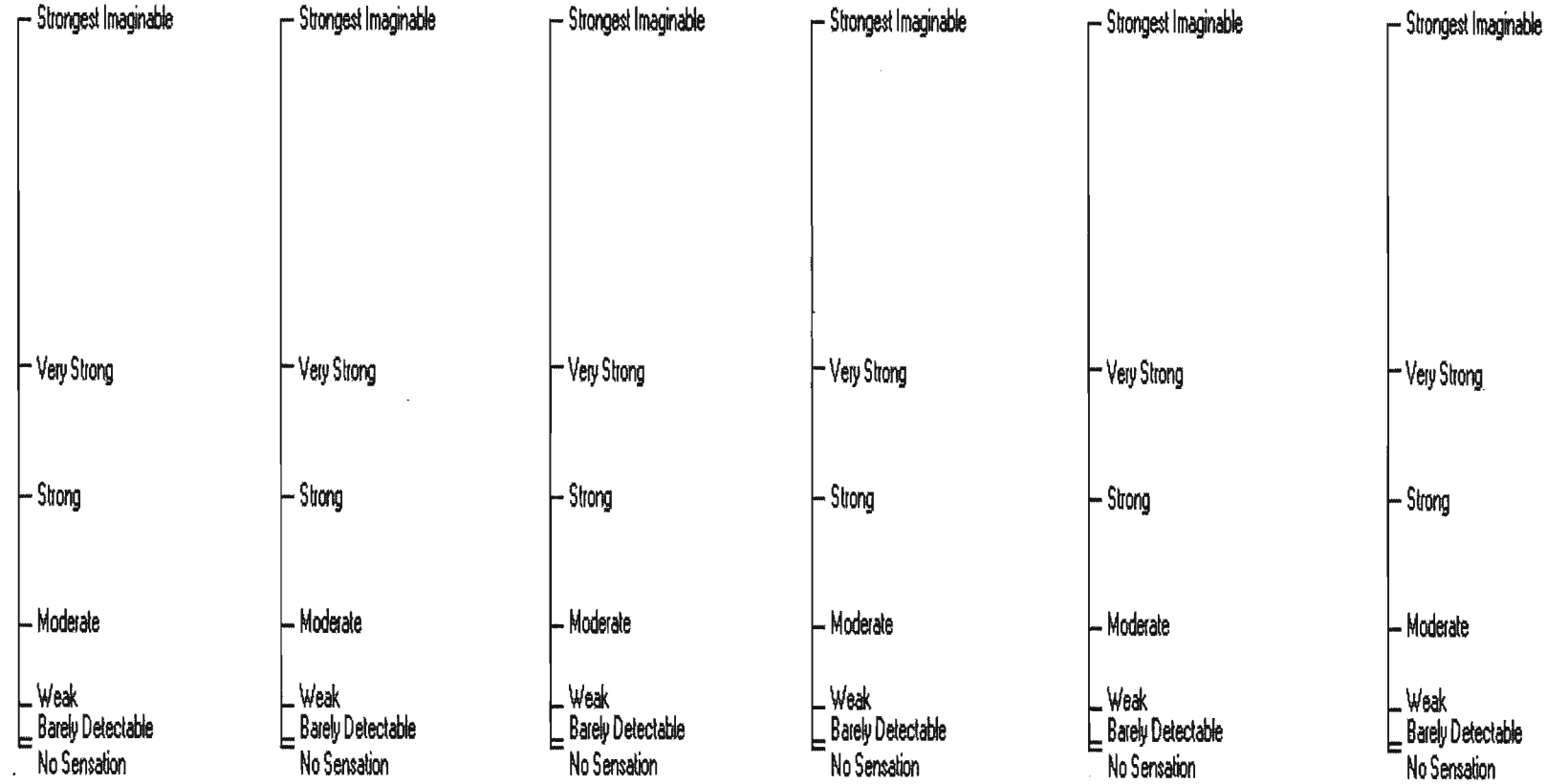
It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing.


Strongest Imaginable
Very Strong
Strong
Moderate
Weak
Barely Detectable
No Sensation
COLD
SWEET
SALTY
SOUR
BITTER
OTHER
SPECIFY:

You are being asked to rate the intensity the sensations you experienced upon **heating of your tongue** by indicating where it lies on a scale of all possible sensations. The scale contains commonly used terms like weak and strong, and the top of the scale is the strongest sensation of any kind that you can imagine experiencing.

When you make your ratings you should use the terms just as you would in daily life. But do not limit your ratings to the terms themselves. A good strategy is to first decide which term most closely describes the strength of the sensation, and then fine-tune your rating by moving your line between that descriptor and the next most appropriate one. For example, if you think a sensation is about moderate, but a little bit stronger, you should place a line on the appropriate place just above moderate.

It is important to note that the top of the scale is "strongest imaginable", which represents the most intense--and therefore most painful--sensation that you can ever imagine experiencing.


Strongest Imaginable
Very Strong
Strong
Moderate
Weak
Barely Detectable
No Sensation
HEAT
SWEET
SALTY
SOUR
BITTER
OTHER
SPECIFY:

APPENDIX K: CHAPTER 4 QUESTIONNAIRE

Demographics

Age: _____

Gender:

F ☐ M ☐

If you are a female, please indicate the first day of your last period (yymmdd).

_____

If you are no longer menstruating, please approximate the time since your last period (for weeks please include a 'w' after your answer, for months a 'm', and for years a 'y')

_____

Ethnicity: Please check the one that best applies, if none of these apply, please indicate your ethnicity under 'Other'. If you would like to add any additional information (e.g., mixed ethnicity), please do so under the 'Comments' section):

White ☐
Chinese ☐
South Asian ☐
Southeast Asian ☐
Black ☐
Filipino ☐

Japanese ☐
Latin American ☐
Arab ☐
Aboriginal ☐
(i.e., North American Indian/Metis/Inuit) ☐
Other (please specify): ☐

_____

Were you born in North America:

Yes ☐ No ☐

If no, where were you born:

City: _____
Country: _____

How many countries outside of Canada and the United States of America (which includes Alaska & Hawaii) have you visited?

0 ☐
1-2 ☐
3-5 ☐
>5 ☐
>10 ☐

Do you consume alcoholic beverages?

Yes ☐ No ☐ If not, why not:

On average, how many times a month do you drink the following beverages?
(please check the appropriate box)

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20-24	25-29	>/=30
white wine																							
red wine																							
beer																							
spirits																							
other																							
(e.g. cooler)																							

On days when you drink the following alcoholic beverages, how many do you consume
(i.e., average # per day), where (**1 drink** = 12 oz. (bottle) Beer OR 6 oz. Wine OR 1.5 oz. Spirit)

	#
white wine	
red wine	
beer	
spirits	
other	
(e.g. cooler)	

Do you smoke, or use tobacco products?

Yes ☐ No ☐

If yes, please indicate how many times a month you use the following tobacco products.
(please check the appropriate box)

	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20-24	25-29	>/=30
cigarettes																							
cigars																							
pipe																							
snuff																							
chewing tobacco																							

On days when you use the the following tobacco products, how many do you smoke/ingest (i.e., average # per day)

	#
cigarettes	
cigars	
pipe	
snuff	
chewing tobacco	